An Exploration of the Relationships between Autism Spectrum Disorders, Theory of Mind, and the Serotonin Transporter Promoter Length Polymorphism

Katie Hamilton
HMLKAT002

ACSENT Laboratory
Department of Psychology
Faculty of Humanities
University of Cape Town
April 2014

A minor dissertation submitted in partial fulfilment of the requirement for the award of the degree Masters of Clinical Neuropsychology

Compulsory Declaration: This work has not been previously submitted in whole, or in part, for the award of any degree. It is my own work. Each significant contribution to, and quotation in, this dissertation from the work, or works, of other people has been attributed, and has been cited and referenced.

Acknowledgements

This study was made possible through funding by the National Research Foundation Thuthuka Grant to Dr Susan Malcolm-Smith.

Thank you to University of Cape Town funding, National Research Foundation and the Harry Crossley Foundation for their generous support throughout my Masters degree.

Thank you to all the children, parents, teachers, and school psychologists for your time and participation in this study. Your participation is key to helping the University of Cape Town's Autism Research Group's endeavours to improve our understanding of Autism Spectrum Disorders, and hopefully translate this knowledge into improved diagnostics and interventions for these families.

Thank you to Dr Colleen O'Ryan and her team at the Department of Molecular and Cell Biology, University of Cape Town. Thank you for the hours spent researching protocols, running samples, and helping to make the genetic aspects of this study possible.

Thank you to my parents, Mike and Elma Hamilton, for always supporting my dreams.

Finally, to Dr Susan Malcolm-Smith: from the moment when this protocol was nothing more than a fleeting idea, to the day the final thesis was submitted, thank you for your unwavering support and guidance.

.

Table of Contents

List of Figures

List of Tables

Abstract

Autism Spectrum Disorder (ASD) is a highly heritable prevalent pervasive developmental disorder. All cases have deficits in social communication and interaction and in restricted and repetitive behaviours and interests. The mechanisms underlying different clinical presentations remain elusive. Deficits in Theory of Mind (ToM), the ability to understand that others have mental states independent of one's own, have been suggested as a possibly underlying the social deficits in ASD. The serotonin transporter promoter length polymorphism (5-HTTLPR) has been implicated in ASD, and as serotonin is implicated in social functioning more generally, it is possible that 5-HTTLPR could underlie social functioning in ASD. As such, ToM and 5-HTTLPR have been implicated in ASD, and specifically as underlying the social deficits typical of this disorder.

This protocol assessed core ASD symptoms (i.e. deficits in social communication and interaction, and impairment in restricted and repetitive behaviours and interests) in 69 children with ASD between the ages of 7 and 14 years. The Autism Social Skills Profile, Social Communication Questionnaire, and Repetitive Behavior Scale – Revised assessed these symptoms. 5-HTTLPR genotypes were established for 55 of these children. ToM was comprehensively assessed in 57 of the children using the University of Cape Town Autism Research Group's Theory of Mind Battery.

This protocol is the first is a series of studies assessing the biological bases for social deficits in ASD. One of the main aims was to pilot the use of ASD scales in a local sample. The preliminary analyses assessed the performance of these scales. This data was also used to assess whether the new DSM-5's merging of social communication and social interaction into a single domain was supported. Study One then assessed for possible relationships between 5-HTTLPR and cores ASD symptoms, and hypothesised that the 5-HTTLPR genotype with the most reduced serotonergic transmission would relate to increased deficits in social communication and interaction. Study Two explored possible relationships between core ASD symptoms and ToM, and between 5-HTTLPR and ToM. It was expected that impairment in social communication and interaction would correlation with reduced ToM ability, and that ToM would be most impaired in children with the genotype with the most reduced serotonergic transmission.

Preliminary analyses found the scales did not perform well in a local sample. This was likely due to cultural, socio-economic, and educational factors. The bluntness of the scales

and broad nature of ASD characteristics likely also contributed. The DSM-5's diagnostic criteria were supported.

Study One and Study Two found no relationships between core ASD symptoms, ToM, and 5-HTTLPR. Core ASD symptoms were assessed very broadly and it was not possible to establish clear phenotypes for the participants, which likely undermined analyses. At most this protocol showed that broad assessment of core ASD symptoms is not specific enough to reveal relationships to underlying mechanisms, and that ToM and 5-HTTLPR are not implicated when board measures are used.

We emphasise the need for better measures in ASD. We also believe the serotonin system needs to be investigated beyond 5-HTTLPR in ASD.

Introduction

Autism spectrum disorder (ASD) is very heritable pervasive developmental disorder (Centers for Disease Control and Prevention, 2012; Kendler, 2010; Kogan et al., 2009). ASD is characterised by deficits in core symptom domains: impaired social communication and interaction; and restricted and repetitive behaviours and interests (American Psychiatric Association, 2013). The cause of ASD has remained elusive, and the exact mechanism(s) underlying different clinical presentations is just as unclear. ASD is likely to result from multigene interactions, so there is a need to investigate the mechanism(s) behind different clinical presentations, and to investigate the genetic contributions to these mechanisms and presentations (American Psychiatric Association, 2013; Kendler, 2010). ASD is highly heterogenous, but as all cases have deficits in core symptom domains, the mechanisms underlying these should be the initial focus of exploration with regard to phenotyping.

Theory of Mind (ToM) deficits have been proposed as possibly underlying the social deficits in ASD. Theory of Mind is the ability to understand that others have mental states that influence their behaviours, and that these mental states are different from one's own mental states (Baron-Cohen, Leslie, & Frith, 1985; Hoddenbach et al., 2012). Although deficits in ToM have been found, what causes them and how they relate to different ASD presentations is still unclear.

The serotonin transporter promoter length polymorphism (5-HTTLPR) has been suggested as a candidate gene for ASD. Serotonin has long been implicated in ASD, and 5-HTTLPR mediates the efficacy of serotonin transmission in the central nervous system (Adamsen, Meili, Blau, Thöny, & Ramaekers, 2011; Arieff, Kaur, Gameeldien, van der Merwe, & Bajic, 2010). Studies have directly implicated 5-HTTLPR genotypes in different ASD presentations (Brune et al., 2006; Tordjman et al., 2001).

Studies implicate specific 5-HTTLPR genotypes in social communication and interaction deficits in ASD, although the nature of these relationships is unclear (Brune et al., 2006; Tordjman et al., 2001). ToM is also implicated in this core symptom domain. It is possible that relationships may not only exist between 5-HTTLPR and ASD, and ToM and ASD, but directly between 5-HTTLPR and ToM as well.

The current protocol is the first in a series exploring underlying biological mechanisms for the deficits in social functioning in ASD. This protocol will pilot the approach of assessing core ASD symptoms across genotypes in a local sample, as well as the suitability of international ASD measures in this sample.

The relationships between core ASD symptoms, ToM, and 5-HTTLPR genotypes are therefore of interest, and were explored in the current protocol.

Review of the Literature

Autism Spectrum Disorder

ASD refers to a group of complex neurodevelopmental disorders that vary markedly in their severity and nature of impairments (American Psychiatric Association, 2013; Rapin & Dunn, 2003). All cases are characterised by persistent deficits in social communication and interaction, and the presence of restricted and repetitive behaviours, interests and activities (American Psychiatric Association, 2013). ASD represents a relatively new diagnosis, as it was only in 1943 that an American Psychiatrist recognised the existence of ASD, and his work laid the foundation for our current understanding of this disorder (Kanner, 1943).

Core diagnostic features of ASD. ASD is a complex disorder and the wide range of possible presentations can make it difficult to characterise. However, all individuals with ASD display some impairment in social interaction and communication, and display a degree of restricted and repetitive behaviours and / or interests (American Psychiatric Association, 2013). These two symptom domains are regarded as independent of one another and each individual can present with different levels of impairment in either domain, which is why such a wide spectrum of presentations exist. For example, one individual may present with markedly worse communication ability than impairment in restricted and repetitive behaviours and interests, while another's social ability may seem far better in relation to their restricted interests or repetitive behaviours. Further, there are several different possible presentations for each core symptom domain, and examples of these variations within each core symptom domain are discussed below. Despite the various possible presentations, deficits in core ASD symptoms are evident across all presentations and serve as the unifying element of ASD. A systematic review of the range of symptom presentations is not possible; however, these core symptom domains are briefly discussed below.

Social communication and interaction in ASD. One of the most striking features of ASD can be the impairment in areas of social communication and interaction (American Psychiatric Association, 2013; Macintosh & Dissanayake, 2006; Prior et al., 1998). Children with ASD often have an impaired ability to relate to others, an impaired ability to understand reciprocal social interactions, and a decreased or absent desire to enter social interactions (American Psychiatric Association, 2013). Leo Kanner (1943) referred to "autistic aloneness"

as the central symptom in the children he studied. “Autistic aloneness” is the psychological state of profound disconnection and separation from others. Kanner (1943) described the children he saw as being unable to relate themselves to other people and other situations from the very beginning of life. As with all aspects of ASD, these deficits vary in severity across cases.

Children on the spectrum often not only withdraw from social participation, but may tend to disregard, ignore, and seem to completely shut themselves off from these situations (Kanner, 1943). In many cases this social aloofness is not a preference but seems to be a failure to develop a social interest during development. In other cases children may struggle to form friendships as their interaction with others is often awkward and they may exhibit socially maladaptive behaviours (American Psychiatric Association, 2013).

As young infants, children on the autism spectrum may fail to exhibit anticipatory postures in order to prepare to be picked up, suggesting a failure even at that age to understand the basic intentions of others (Happé & Frith, 1996; Klin, Volkmar, & Sparrow, 1992). As they age, they may demonstrate unusual body postures and gestures in social situations, as well as inappropriate facial expressions (American Psychiatric Association, 2013). Many struggle to read the non-verbal cues and body language of others, and often respond inappropriately. Failure to initiate or maintain eye contact often becomes more evident as these children age, and difficulties in joint attention mark their social isolation when surrounded by others (Meyer & Minshew, 2002). Their lack of understanding of others inevitably translates into incomprehension of social norms and conventions. Difficulties understanding others can include difficulties with Theory of Mind. Current research suggests impaired Theory of Mind may be a significant aspect of ASD, and this is discussed later.

Children with ASD often only develop shallow friendships that are inappropriate for their age, or they simply fail to develop friendships at all (American Psychiatric Association, 2013). Some may develop strategies to navigate social situations, and these strategies can become more complex and refined with age, but even then they often remain restricted. The social behaviour of these individuals may therefore come across as scripted and unnatural. Social competence is often further hindered by impaired language ability.

Impaired language in ASD. Communication deficits in ASD can present as impairments in the expression and/or comprehension of language (American Psychiatric Association, 2013; Zillmer, Spiers, & Culbertson, 2008). Language deficits represent one aspect of a more general deficit in verbal and non-verbal communication (Verhoeven et al., 2012a). The ability to communicate with gestures is regarded as a precursor to the

development of language and the skills needed for social interactions, yet children whose ASD includes impaired language often also fail to utilise non-verbal behaviours to communicate.

Language is fundamentally intertwined with cognitive, social, and emotional domains of function, and these areas develop in ways that involve ongoing influencing of one another (Mody et al., 2013). The development of language therefore significantly affects children's thinking, learning, and their ability to form relationships. As children on the spectrum do not orient to speech from a young age, including when their names are called, it is likely that communication impairment may be secondary to their deficits in social competence and reciprocity (Mody et al., 2013; Verhoeven et al., 2012b). Despite the intertwined nature of language deficits with other domains, language impairment is in itself a striking feature of ASD, and it poses a significant challenge to the quality of life that these individuals can obtain.

Language development varies in children with ASD. Approximately 25% of individuals with ASD fail to develop functional language (Mody et al., 2013). The remaining children may show delayed but otherwise normal development, and some display typical language development. Moreover, when language is present, it is often unusual (Zillmer et al., 2008). It can be characterised by stereotyped content, its content can be tangential, or responses to questions can be inappropriate or unrelated to the context of the conversation. Tracking of developing language in children with ASD shows that instead of generating their own sentences, they may simply repeat sentences they have heard (sometimes even appropriately) but only after a significant time gap. In severe cases these children can exhibit echolalia (Rapin & Dunn, 2003). Pronoun misuse is common and is most often noted in a failure to switch between pronouns referring to the self versus those referring to others (Happé & Frith, 1996). Children on the spectrum often have a very literal understanding of language, and may therefore fail to use generalised terms. Thus, some struggle to understand the general uses of 'yes' or 'no', and resort to responding to questions by repeating them as statements. Difficulties with nonliteral language can also result in an inability to understand sarcasm and irony (Happé, 1993; Meyer & Minshew, 2002).

Ultimately, these language deficits impair a child's ability to request information, to comment on events, or to clearly assert their own desires and needs. Language impairment further isolates these children who already exhibit such profound isolation.

Restricted and repetitive behaviours and interests in ASD. The second central feature of ASD is restricted and repetitive behaviours and interests (American Psychiatric

Association, 2013; Lam, 2004; Zillmer et al., 2008). This symptom domain consists of a motor component and / or preoccupations.

The motor component of this symptom domain can range from harmless behaviours, such as arm flapping, to tic-like behaviour, such as counting, to self-injurious behaviours that include head-banging (American Psychiatric Association, 2013; Lam, 2004; Szatmari et al., 2006). These behaviours can become worsened during periods of anxiety. Restricted sensory and motor behaviour is more common in lower functioning children with ASD, while insistence on sameness and/or preoccupations with very specific subjects is more frequently seen in higher functioning children who have better language abilities (Szatmari et al., 2006).

Some children on the autism spectrum may have preoccupations with very specific subjects, with certain categories of objects, with specific qualities of objects, or with the movement of objects (American Psychiatric Association, 2013). This focused attitude often extends into daily activities, with children insisting on sameness in their environment and routine (American Psychiatric Association, 2013; Howlin, 2003; Lam & Aman, 2007; Macintosh & Dissanayake, 2006; Prior et al., 1998; Szatmari et al., 2006). This can include developing elaborate routines that must be followed. Insistence on consistent routines includes the timing and sequencing of events, as well as how the activities are conducted. An example would be that the daily drive to school must follow the same route, at the same time, after the same sequence of morning activities. These children may also insist on order, and can spend hours sorting or lining up their toys rather than playing with them. The insistence on sameness in the environment includes the physical environment, such as the layout of furniture in a room, but may also include the people in the child's environment, such that changes in appearance of a parent, sibling, or teacher can be very upsetting. Attempts to interfere with a child's repetitive and restricted behaviours or interests can be met with distress or rage.

Each symptom domain in ASD has a variety of possible presentations that could not be comprehensively discussed in this literature review. As such, only a brief overview with limited examples was provided. The above descriptions do not, and cannot, fully capture the diversity of clinical presentations seen in ASD. Further, children with ASD do not only present with deficits in these core symptom domains, but often present with further associated characteristics and comorbid disorders.

Other common features of ASD. ASD has many associated features that can be used to support a diagnosis, although it is important to note that these signs and symptoms are not unique to ASD (American Psychiatric Association, 2013).

Motor symptoms are often present (American Psychiatric Association, 2013; Lam & Aman, 2007; Manjiviona & Prior, 1995; Noterdaeme, Wriedt, & Höhne, 2010; Szatmari et al., 2006; Thede & Coolidge, 2007). These symptoms can be present in the form of clumsiness or odd gait, with some children who only walk on their toes. Some motor symptoms are classified under restricted and repetitive behaviours, such as arm flapping, while others are characterised as self-injurious behaviours, such as head-banging. Individuals may present with catatonic-like symptoms, but this is rare.

Unusual sensory features have been noted since the original descriptions of ASD (Kanner, 1943). These can include sensory fascinations or alterations in sensory sensitivities (American Psychiatric Association, 2013; Brock et al., 2012; Fung, Chahal, Libove, Bivas, & Hardan, 2012; Liss, Saulnier, Fein, & Kinsbourne, 2006). These sensory features range from apparent indifference to over-sensitivity. Children with less sensitivity can appear indifferent to pain, and their heightened threshold to stimulation can lead to sensation seeking and a need for greater stimulation. In contrast, children who are over-sensitive may be intolerant of stimulation and may require active intervention, such as wearing noise-cancelling headphones, to prevent adverse reactions to normal levels of stimulation.

Children with ASD can present with a variety of sleep difficulties, including slow sleep onset, irregular sleep-waking patterns, many night awakenings, and early waking (Gabriels, Cuccaro, Hill, Ivers, & Goldson, 2005; Krakowiak, Goodlin-Jones, Hertz-Picciotto, Croen, & Hansen, 2008; Richdale, 1999; Schreck, Mulick, & Smith, 2004; Springer, van Toorn, Laughton, & Kidd, 2013). Poor sleep can aggravate existing symptoms, and can lead to further difficulties in concentration, school performance, and behaviour.

Gastrointestinal symptoms, dietary sensitivities and difficulty regarding food intake are common in children with ASD (Cermak, Curtin, & Bandini, 2013; Chandler et al., 2013; Knivsberg, Reichelt, Hoien, & Nodland, 2002; Kral, Eriksen, Souders, & Pinto-Martin, 2013; Talay-Ongan & Wood, 2000; White, 2003). These children can be very selective about what food they are willing to eat and are often unwilling to try new or unfamiliar foods (Cermak et al., 2013; Kral et al., 2013; Talay-Ongan & Wood, 2000). Unusual eating patterns and odd behaviour with food, such as touching and smelling without eating the food, can be present, as well as the ingestion of non-food substances. Some researchers have ascribed these difficulties with food to sensory sensitivity, where the textures, smells and tastes of the foods may be why the children are either resistant or, in some cases, overly fascinated with certain foods (Cermak et al., 2013; Talay-Ongan & Wood, 2000). Gastrointestinal symptoms occur more frequently in children with ASD than in typically developing children, and include

vomiting, diarrhoea, abdominal pain, bloating, and constipation (Cermak et al., 2013; Chandler et al., 2013; Kral et al., 2013; White, 2003). Food sensitivities and gastrointestinal symptoms combine to create constant challenges in daily eating routines. The limited food intake that results from this may cause inadequate nutrition, hinder development, and exacerbate other ASD symptoms (Cermak et al., 2013; Knivsberg et al., 2002; Kral et al., 2013; White, 2003).

Common comorbidities. Children with ASD may also receive comorbid diagnoses (American Psychiatric Association, 2013). Intellectual impairment is one of the most common comorbid diagnoses (American Psychiatric Association, 2013; Centers for Disease Control and Prevention, 2012; Chakrabarti & Fombonne, 2005; Happé & Frith, 2006; Y. S. Kim et al., 2011; Thede & Coolidge, 2007). Intellectual ability can range from severely impaired to above average. However, even individuals with high intellectual functioning can present with a substantial difference between adaptive functional skills and intellectual ability.

Anxiety is very common in ASD, and as these individuals reach adulthood they may be diagnosed with co-morbid depression or anxiety disorders (American Psychiatric Association, 2013; Simonoff et al., 2008). Attention Deficit/Hyperactivity Disorder (American Psychiatric Association, 2013; Peacock, Amendah, Ouyang, & Grosse, 2012; Simonoff et al., 2008) and seizures or Epilepsy (American Psychiatric Association, 2013; Bolton et al., 2011; Peacock et al., 2012; Simonoff et al., 2008) are also frequently diagnosed in children with ASD.

The heterogeneous nature of ASD, as well as the possible associated characteristics and comorbidities, can make it a challenging disorder to characterise and diagnose. The high prevalence of ASD globally emphasises the need to further improve our understanding of the disorder, as well as our current diagnostic procedures and interventions. The prevalence of ASD is discussed below, followed by a discussion of recent changes in ASD diagnostic criteria; changes that impact our understanding of ASD moving forward.

Prevalence of ASD. ASD is one of the most prevalent developmental disorders. Although the prevalence of ASD is unknown in South Africa, internationally it ranges from 27.5 to 77.2 per 10 000, with one study finding a prevalence of 110 per 10 000 in an American population (Baird et al., 2006; Centers for Disease Control and Prevention, 2012; Kogan et al., 2009). ASD is predominantly diagnosed in males, with the ratio of male to female diagnoses being 4:1. The prevalence of ASD does not appear to be affected by race or ethnicity, but research indicates that communities of low socio-economic status are likely to under-identify cases of ASD, and that diagnoses from these areas tend to be made at a later

age (Bertrand et al., 2001; Fombonne, 2003; Malcolm-Smith, Hoogenhout, Ing, Thomas, & de Vries, 2013).

The high prevalence of ASD is concerning and there is an increasing global awareness of the burden of this disorder (Baird et al., 2006; Bertrand et al., 2001; Boyle et al., 2011; Y. S. Kim et al., 2011; Kogan et al., 2009; Malcolm-Smith et al., 2013; Rice, 2009; J. G. Williams, Higgins, & Brayne, 2006). The heterogeneous nature of the disorder means different individuals have varying needs for support, and many of these children will never develop into independently functioning adults. This places substantial strain on families and the state. There is generally very little information available locally on ASD and its social and monetary cost (Malcolm-Smith et al., 2013). South Africa also lacks sufficient diagnostic and intervention services, meaning that even when a formal diagnosis is made, very limited services are available to the child and their family.

ASD diagnostic criteria. The ASD diagnostic criteria were updated with the recent release of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American Psychiatric Association, 2013). An ASD diagnosis is made if an individual has "persistent deficits in social communication and social interaction across multiple contexts", has persistent "restricted, repetitive patterns of behaviour, interests, or activities", and if these symptoms were present in early development and cause significant impairment in daily living. These symptoms fall on continuums, with some individuals showing mild to moderate symptoms, and others presenting with far more severe symptoms. Although deficits may be less obvious in some situations and aggravated in others, they must be present to some degree across multiple contexts. Individuals can develop compensatory mechanisms that mask these symptoms, so a diagnosis can be made if the current presentation causes significant impairment and diagnostic criteria are met by a combination of current symptomatology and historical information.

The shift from the DSM-IV-TR to the DSM-5. The DSM-5 included two major changes from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revised (DSM-IV-TR) criteria. The first was the discarding of DSM-IV-TR ASD subtypes, and the second was the merging of the previously separate core symptom domains of social communication and social interaction.

The discarding of ASD subtypes according to the DSM-IV-TR. The DSM-IV-TR subcategorised ASD into separate pervasive developmental disorders that included autistic disorder ('autism' hereafter), Asperger's disorder ('Aspergers' hereafter), pervasive developmental disorder not otherwise specified (PDD-NOS), Rett's disorder, and childhood

disintegrative disorder (American Psychiatric Association, 2000). Intellectual ability was used to further divide autism diagnoses into high functioning autism and low functioning autism, depending on whether the child's IQ was above or below 70 points, respectively.

These subtypes featured wide variations in symptom severity, functional disability, and in intellectual ability (Frazier et al., 2012; Geschwind, 2009). With the release of the DSM-5, these diagnoses have been discarded and replaced by ASD as an umbrella diagnosis with specifiers to indicate the key symptoms and deficits of each individual (American Psychiatric Association, 2013; Huerta, Bishop, Duncan, Hus, & Lord, 2012). Varied presentations are now differentiated through the use of specifiers, so that one can be diagnosed with ASD with or without: intellectual impairment; language impairment; a medical or genetic condition or environmental factor; another neurodevelopmental, mental, or behavioural disorder; or, catatonia. The age of first concern, severity of symptoms, and whether a regression of skill(s) occurred are also specified. Therefore, despite discarding the separate diagnoses of the DSM-IV-TR, the DSM-5 is still able to individualise diagnoses. These revised diagnoses aim to provide a richer description of the individual's clinical presentation that is more accurate than the DSM-IV-TR subtypes. It is also hoped that this approach will be more medically and scientifically useful by being able to correctly classify individuals according to ASD phenotypes (Huerta et al., 2012).

This shift in the conceptualisation of ASD is substantial and one needs to understand why the dimensional approach was chosen over the previous categorical approach. The main reason was that the differences between subtypes were frequently found to be quantitative, as individuals from different subtypes all shared impairment in the core ASD symptoms, but to different degrees (Macintosh & Dissanayake, 2006; Manjiviona & Prior, 1995; Meyer & Minshew, 2002; Miller & Ozonoff, 2000; Witwer & Lecavalier, 2008). As such, categorical differences between subtypes were often difficult to identify, leading to situations where a child could meet the diagnostic requirements for more than one ASD subtype. Although the DSM-IV-TR catered for this by having an Autism diagnosis take precedence over other subtype diagnoses, it led to questions regarding whether qualitative differences existed between the subtypes.

Moreover, research on whether autism and Aspergers were different disorders indicated that they may not have been, especially in the case of high functioning autism and Aspergers (Eisenmajer et al., 1996; Howlin, 2003; Mayes, Calhoun, & Crites, 2001; Noterdaeme et al., 2010; Prior et al., 1998; Swedo, Thorsen, & Pine, 2008; Thede & Coolidge, 2007; K. Williams et al., 2008). Diagnostically these disorders were differentiated

only by the presence of a developmental language delay in autism (American Psychiatric Association, 2000). However, the presence or absence of a delay in language is not a reliable indicator of etiology or future outcome (Howlin, 2003). Further, although the DSM-IV-TR required language delay for an autism diagnosis, it did not stipulate a difference in the degree of impairment in social communication between autism and Aspergers (American Psychiatric Association, 2000). The distinguishing difference was purely based on history rather than current ability.

Further, research showed that social communication and verbal skills seemed to equalise between these groups with age and that the difference could disappear completely by late childhood or adulthood (Howlin, 2003). It was therefore likely that children would shift from high functioning autism diagnoses to Aspergers' diagnoses as they aged (American Psychiatric Association, 2000; Howlin, 2003). Due to the similarities in etiology, outcome, and the evidence that adult presentations of these disorders often lacked distinction, there was a risk that Aspergers diagnoses were largely invalid.

Similarly, the validity of PDD-NOS was called into question. This diagnosis had no defining feature to differentiate it from autism and Aspergers, but was rather a default diagnosis for the absence of those disorders (Huerta et al., 2012; Matson & Boisjoli, 2007; Prior et al., 1998; Witwer & Lecavalier, 2008). The DSM-IV-TR listed PDD-NOS as an ASD subtype, but did not include diagnostic criteria (American Psychiatric Association, 2000). It was generally accepted that PDD-NOS was diagnosed when the symptom cluster presented as a 'mild' or 'borderline' autism, but there was no consensus on specific, reliable diagnostic criteria (Huerta et al., 2012; Matson & Boisjoli, 2007). There was little reason or evidence to indicate that PDD-NOS was qualitatively different from other ASD subtypes.

Rett's disorder was the only ASD subtype that was categorically unique. Rett's disorder has an established cause: a genetic mutation that is not present in other ASD cases (Amir et al., 1999). This genetic cause differentiated Rett's disorder from other DSM-IV ASD subtypes and also indicated that it should be reclassified as a medical condition and removed from the DSM completely (Swedo et al., 2008). In response to this, Rett's disorder does not fall under ASD in the DSM-5, but can be specified as a co-morbid disorder alongside ASD (American Psychiatric Association, 2013).

Thus, the ASD subtypes listed in the DSM-IV-TR were argued by many to be invalid as they were distinguished by the degree of deficit rather than by qualitative differences. It was considered more appropriate to conceptualise cases on a dimension of symptom severity. A study by Huerta, Bishop, Duncan, Hus and Lord (2012), which is the largest, most up-to-

date and most comprehensive assessment of the DSM-5 ASD criteria, used symptom extraction from previously collected data and found the DSM-5 criteria to be more accurate and reliable than the DMS-IV-TR criteria. There is, however, considerable controversy around the changes in the diagnostic criteria, with some clinicians and researchers opposing the abandonment of subtypes, the merging of social communication and social interaction symptom domains, and suggesting that other significant characteristics should have been integrated into the diagnostic criteria (Boomsma et al., 2008; Ghaziuddin, 2010; Matson, Hattier, & Williams, 2012; Wing, Gould, & Gillberg, 2013)

The merging of social communication and social interaction into a single symptom domain. The DSM-IV-TR stipulated that a child had to present with qualitative impairment in social interaction as well as in communication (American Psychiatric Association, 2000). These domains were considered independent of one another so a diagnosis required specific impairment in both areas. However, as was discussed previously, language and communication ability are informed by one's cognitive ability, social experiences, and emotional functioning (American Psychiatric Association, 2013; Mody et al., 2013; Verhoeven et al., 2012a). The separation of observed communication ability from one's level of social interaction was argued to be artificial. Non-verbal children often fail to compensate for the lack of speech with non-verbal communication – that is, they simply do not interact with others in a social way (Mody et al., 2013). In such cases, one cannot disentangle the social disinterest from the failure to communicate.

Several studies have explored whether social interaction and social communication in ASD can be separated through statistical analyses, but found that models that merge these symptoms are stronger (Boomsma et al., 2008; Frazier, Youngstrom, Kubu, Sinclair, & Rezai, 2008; Mandy, Charman, & Skuse, 2012; Snow, Lecavalier, & Houts, 2009; van Lang et al., 2006)**.** These studies have used goodness-of-fit analyses (Boomsma et al., 2008; van Lang et al., 2006) and confirmatory and exploratory factor analysis (Frazier et al., 2008; Snow et al., 2009). Further, it was noted that the DSM-IV-TR model which separated social interaction and social communication had empirical identification problems due to improper estimated correlation between these two domains (Boomsma et al., 2008).

The merging of the two symptom domains is thus supported theoretically and practically (through statistical analyses), and is regarded by some as showing the progress that has been made in looking beyond the surface presentation of symptoms and understanding the underlying deficits (Huerta et al., 2012).

To date, the underlying mechanisms behind ASD have been elusive, but perhaps these shifts in diagnostic criteria will lead to greater advances in understanding contributing factors.

Theory of Mind

Deficits in Theory of Mind (ToM) have been proposed as an underlying mechanism for the social impairment seen in ASD. ToM refers to one's ability to understand that other people possess their own thoughts, feelings, and beliefs, that these states in others are independent of oneself, and that these mental states influence other people's behaviour (Baron-Cohen et al., 1985). ToM underlies the ability to understand social situations and predict others' actions, making it crucial for adaptive social functioning (Leslie, 1987). Social competence requires social reciprocity, which depends on the ability to understand that others' mental states affect their actions, and that these mental states may be different from one's own – that is, it depends on ToM. Studies on typically developing children have confirmed this positive correlation between social competence and ToM (Bosacki & Wilde Astington, 2001; Repacholi & Slaughter, 2003).

Theory of Mind development in typically developing children. The development of ToM has been tracked in typically developing children up until the age of eleven, and a set developmental trajectory has emerged (Wellman, Cross, & Watson, 2001; Wellman & Liu, 2004). At 14 to 24 months children engage in pretend play and show joint attention (Frith & Frith, 2003). At two years children spontaneously begin to talk about their mental states and show an understanding of other people's desires (Wellman & Woolley, 1990). Between three and five years old children begin to appreciate the difference between appearance and reality, and to understand their own and others' false beliefs (Bibby & McDonald, 2005; Naito, Komatsu, & Fuke, 1994b). Children then begin to understand second-order beliefs – beliefs about other people's beliefs – between five and seven years old. Between six and ten years of age, children develop understanding of language forms such as metaphors, sarcasm, and irony, as well as the ability to differentiate between lies and jokes (Ackerman, 1981; Brüne & Brüne-Cohrs, 2006; Pollio & Pollio, 2008). The ability to recognise social faux pas develops between nine and eleven years of age (Baron-Cohen, O'Riordan, Stone, Jones, & Plaisted, 1999).

Theory of Mind deficits in ASD. ToM deficits in ASD can be seen across development – from the initial absence of pretend play, to the later inability to understand the intentions and behaviours of others. These deficits may represent a core area of deficit that characterises this disorder (Hoddenbach et al., 2012).

ToM deficits present on a dimension of severity. In general, these children struggle to understand differences between appearance and reality. Problems with pretend, imaginative or symbolic play include the failure to initiate these types of play, or only engaging in them minimally. When play is entered into, children will often resort to repetitive actions that illustrate a lack of comprehension of the symbolic meaning of toys (Zillmer et al., 2008). As language develops, these children struggle to recognise mental state words such as "think" and "know", and do not understand non-literal speech such as irony and metaphor (Baron-Cohen et al., 1985; Charman et al., 2000; Happé, 1993).

Formalised tests have confirmed these ToM deficits in ASD (Baron-Cohen et al., 1985; Fombonne, Siddons, Achard, Frith, & Happé, 1994; Hoddenbach et al., 2012). Many children with ASD fail false-belief tasks, but between 15 and 55% are able to pass these tasks (Happé & Frith, 1996; Ozonoff & McEvoy, 1994). Those who are able to pass false-belief tasks tend to have higher verbal IQs, and Happé (1995) found that ASD children needed a verbal mental age of 11 years 9 months to pass first-order false-belief tasks. As mentioned above, typically developing children start to understand false-beliefs between three and five years of age. Children with ASD who do develop ToM therefore show a substantial delay in this development.

The reason for these deficits remains unclear, and they cannot be explained by cognitive ability. Hill and McCune-Nicolich (1981) found a poverty of pretend play in children with ASD, but appropriate levels of pretend play in children with mental retardation in the absence of ASD. Baron-Cohen and colleagues (1985) compared ToM test performance between children with ASD, children with mental retardation without ASD, and typically developing children, and they found the children with ASD were significantly more impaired compared to either of the other two groups. It is therefore not clear why ToM would be impaired in these individuals.

ToM deficits may explain the social awkwardness characteristic of ASD, but this remains controversial. ToM task performance may overestimate a child's spontaneous ToM abilities (Hutchins, Prelock, & Bonazinga, 2012; Scheeren, de Rosnay, Koot, & Begeer, 2013; Senju, 2012). Tasks are often presented in a controlled environment and only assess certain behaviours and skills that would otherwise be needed collectively for spontaneous ToM. For instance, in a task a child may only be provided with what a character said in a story, and told to infer from that what the character was thinking. In a real life scenario the child would also be presented with the experience of the situation, the character's facial expression, body language, tone of voice, and their own impression of, and previous experiences with, that

person. The simplified, focused nature of the ToM tasks may therefore not be capable of accurately measuring real life ability.

Findings on the relationships between ToM and other ASD characteristics and abilities have been mixed. Fombonne et al. (1994) used the Vinelands Adaptive Behaviour Scale (VABS) to assess real life competence in an adolescent and adult ASD sample in France. They found that that higher mental age, as indicated by verbal and full IQ scales, and higher chronological age were related to better performance on ToM tasks. The VABS subscale "Maladaptive Behaviours" assessed dysfunctional behaviours in everyday life, and they found that the participants who were better able to understand the mental states of others showed less of these dysfunctional behaviours. A similar study in an ASD sample by Frith et al. (1994) used the VABS and supplemented it with items designed to distinguish between social behaviours that necessitated ToM and those that could be learned. They found that only those participants who could pass false-belief tasks were capable of insightful interactions in daily life. These participants also exhibited better verbal and communication abilities.

More recently, Lerner et al. (2011) assessed ASD symptoms and their relationship to ToM scores on the Theory of Mind Inventory. They found ToM correlated positively with improved social skills, and negatively with autism-related social impairments and ASD symptoms, indicating that deficits in ToM could underlie impairment in ASD. However, a previous study by Joseph and Tager-Flusberg (2005), which examined the whether ToM ability and executive functions could explain the variance in ASD symptom severity, found that ToM did not explain the variance in social interaction or in repetitive behaviour.

Most studies divide participants categorically as those who pass or those who fail false-belief tasks (Scheeren et al., 2013). As mentioned above, ToM is an ability that develops along a dimension. Rating the presence or absence of ToM ability based on the ability to pass one specific point of ToM development (e.g. false-belief reasoning) is an over-simplification of ToM ability and will not allow identification of differences in actual ToM ability. Further, symptom profiles were not established for the groups that attained different levels of success in the ToM tests. It is thus possible the researchers did not recruit comparable samples, and that their samples represented ASD subgroups with different symptom profiles.

Clarity regarding the role of ToM in ASD could be obtained by testing ToM alongside level of impairment in each of the core ASD symptoms to see if variable profiles emerge, rather than simply assessing the relationship to overall ASD severity. Insight into why ToM is impaired in ASD could also be gained through including ToM ability in investigations of

mechanisms underlying ASD. This includes investigations into genetic contributors, such as the candidate gene 5-HTTLPR.

The Serotonin Transporter Promoter Length Polymorphism

ASD is one of the most heritable psychiatric disorders, with some researchers stating a heritability estimate of 80% (Kendler, 2010). This heritability is likely due to multigene interactions, with different genes contributing to different aspects of ASD (American Psychiatric Association, 2013). Several candidate genes for ASD have been proposed, one of which is the serotonin transporter promoter length polymorphism (5-HTTLPR), which mediates transmission of serotonin in the central nervous system. A brief mention of the current directions of genetic research in ASD is discussed below, followed by a more detailed discussion of 5-HTTLPR specifically. A more detailed overview of ASD genetic research is beyond the score of this thesis, and the hundreds of other candidate genes being explored are not immediately relevant to the current study.

ASD genetic research. The genetic basis of ASD has remained elusive, but the high heritability estimate and equally high concordance of ASD between identical twins indicate the importance of research in this area (Kendler, 2010; Muhle, Trentacoste, & Rapin, 2004). Current research focuses on establishing which genes contribute to a predisposition to ASD, which genes contribute to specific symptoms in ASD, and the likely outcomes of interactions between gene products (Poelmans, Franke, Pauls, Glennon, & Buitelaar, 2013). Genome-wide studies have compared the genomes of individuals with ASD to genomes of control subjects and have identified an estimated 400 candidate genes (Cho et al., 2011; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013).

Genetic research on ASD in South Africa is only just beginning, and is therefore very limited. The allelic distribution of 5-HTTLPR has been researched and is discussed below (Arieff et al., 2010; Esau, Kaur, Adonis, & Arieff, 2008). To my knowledge, the only other published local study on genetic and ASD was that by Sharma et al. (2013) which explored the reelin gene (RELN). Reelin is a protein involved in the lamination of the brain during the embryonic period, and is also involved in cell signalling and synaptic plasticity in adult life. Sharma et al.'s (2013) study found a significant association to a specific single-nucleotide polymorphism, rs736707, but not to others, and represented the first report for this genetic association in a South African population. Genetic researchers at the University of Cape Town are currently investigating the role of circadian clock genes and circadian rhythms in the development of ASD. The limited amount of research being conducted locally on the

genetic underpinnings of ASD highlights the importance of development in this area. The current protocol served as a foundation study for further research into the biological bases of social deficits in ASD, and focused on 5-HTTLPR because of serotonin's role in social competence, and because the allelic distribution of 5-HTTLPR has been established in South Africa.

5-HTTLPR. 5-HTTLPR is a variable repeat sequence in the promoter region of the serotonin transporter protein (SERT, 5-HTT). SERT is located on the pre-synaptic membranes of serotonergic neurons (Sen, Burmeister, & Ghosh, 2004). SERT acts to reabsorb serotonin by moving serotonin from the synaptic space back into the presynaptic neuron (Kolevzon, 2006). 5-HTTLPR genotypes moderate the transcriptional efficacy of SERT by determining its different expressions in the pre-synaptic axonic membranes (Adamsen et al., 2011; Arbelle, 2003).

5-HTTLPR variants. 5-HTTLPR has two common alleles (the long (*l*) and short (*s*) alleles) and several rare variants (Huang & Santangelo, 2008). The short allele has reduced transcriptional efficacy, resulting in reduced serotonergic transmission. The short allele reduces the availability of serotonin by approximately 50% (Pérez-Edgar et al., 2011). The long allele has 528 base pairs and contains 16 repeat elements, while the short allele has 484 base pairs and contains only 14 repeat elements. The combinations of these alleles allow for three common genotypes: *l*/*l*, *l*/*s*, and *s*/*s*. Although the frequencies of these alleles differ across populations due to different genetic heritages, the long allele tends to be more common (Arbelle, 2003; Esau et al., 2008; Jacob, 2004; Klauck, Poustka, Benner, Lesch, & Poustka, 1997; Reneman et al., 2006). A local study on healthy South Africans confirmed that the long allele was more common, with the *l*/*l* genotype occurring in 61.40% of their participants, the *l*/*s* genotype occurring in 33.92% of their participants, and then the *s*/*s* genotype occurring in 4.68% of their participants (Esau et al., 2008). In contrast, a local study on individuals with ASD found a significantly higher frequency of short alleles (Arieff et al., 2010). Their sample consisted of 52% *l/l* genotypes, 18% *l/s* genotypes, and 30% *s/s* genotypes. This implicates reduced serotonergic transmission, as mediated by 5-HTTLPR, in ASD. As 5-HTTLPR affects the rate at which serotonin is reabsorbed by the presynaptic neuron, it is implicated in serotonin-related disorders and processes.

Serotonin. Serotonin is a monoamine neurotransmitter (Osborn, 2012; Ropper & Samuels, 2009). The serotonin system originates in the raphe nuclei of the brainstem, and then projects throughout the central nervous system to areas including, but not limited to: the cerebral hemispheres, the diencephalon (that is, the thalamus and hypothalamus), sub-cortical

structures of the limbic system, the cerebellum, and down to the spinal cord. Serotonergic neurotransmission affects sensory processing, and a wide range of behaviours, cognition and emotion processing (Canli & Lesch, 2007; Osborn, 2012).

Dysregulation of serotonin can disrupt social functioning (Canli & Lesch, 2007; Edwards & Kravitz, 1997; Higley et al., 1996; Kruesi et al., 1990; Patkar et al., 2002; Pérez-Edgar et al., 2011). Serotonin is related to social dominance, which serves as a measure of social competence in primates, and serotonin functioning therefore relates to competent social behaviour (Higley et al., 1996). Human studies have noted a relationship between poor serotonin functioning and anxiety. A study that measured serotonin levels in the central nervous system, as measured in cerebrospinal fluid, found that a specific metabolite of serotonin (5-hydroxyindoleacetic acid) was linked to aggression toward others (Kruesi et al., 1990): higher levels of this metabolite, which indicate better serotonergic transmission, were linked to lower aggression toward others while lower levels linked to increased aggression to others. Further, a linear relationship between serotonin availability and attention bias toward angry and happy faces has been found (Pérez-Edgar et al., 2011). Reduced serotonin functioning may then prime individuals toward neurobiological responses to threat, and to information processing strategies biased to perceive threat.

Reduced serotonin transcription, as mediated by 5-HTTLPR genotypes, has shown an association with higher neuroticism and greater harm avoidance (Greenberg et al., 2000; Kruesi et al., 1990). Serotonin is necessary for competent social behaviour, and its dysregulation can result in higher anxiety and neuroticism, greater threat perception and harm avoidance, and increased aggression. Together these maladaptive behaviours can significantly undermine social functioning. Variable transmission rates due to 5-HTTLPR can lead to serotonin dysregulation.

Serotonin and ASD. Serotonin has long been implicated in ASD, although its role in symptom presentations remains unclear. Elevated whole blood levels of serotonin (hyperserotonemia) have been found in approximately one third of ASD cases (Hanley, Stahl, & Freedman, 1977; Kolevzon, 2006) and approximately 25-50% of first degree relatives of individuals with ASD (Burgess, Sweeten, McMahon, & Fujinami, 2006; Cook & Leventhal, 1996). This hyperserotonemia is mostly detected in the platelets and not in the plasma (Cook & Leventhal, 1996), and recent research indicates that serotonin levels in cerebrospinal fluid are not elevated (Adamsen et al., 2011). In addition, ASD shares symptomatology with depression, anxiety disorders, shyness, and social phobias, which are characterised by decreased levels of serotonin (Klauck et al., 1997; Mann, 1999). Decreasing serotonergic

transmission, as is achieved through acute tryptophan depletion, tends to aggravate ASD symptoms (Cook & Leventhal, 1996; McDougle et al., 1996). Together, the difference in serotonin concentration in platelets versus plasma and CSF, and the relationship between reduced serotonin and worsening in ASD symptoms, indicates that although excess serotonin is being produced by some individuals with ASD, the neurotransmission is undermined, so these individuals present with symptoms of insufficient serotonin. This is possibly due to reduced transcriptional efficacy by 5-HTTLPR.

Many selective serotonin-reuptake inhibitors (SSRIs) are specifically designed to target SERT. SSRIs do this by influencing the function of 5-HTTLPR to increase the time serotonin spends in the synaptic cleft (Kim et al., 2000; Kolevzon, 2006). SSRIs are commonly included in ASD treatments, although the evidence regarding efficacy of these drugs is mixed (Kolevzon, 2006). When SSRIs do work, they improve motor skills, verbalisation, and increase communicative gestures (Adamsen et al., 2011; Kolevzon, 2006). Sugie and colleagues (2005) compared the efficacy of SSRIs between ASD individuals with only short alleles (*s/s* genotype) and those that had a long allele (*l/l* and *l/s* genotypes). They used the Behavioural Assessment Scale, which assesses seven areas (facial expression and eye movement, emotion and mood, interest and will, activities, attention, personal relationships, and language) in which individuals could possibly show improvement. They found that the long allele group showed greater global improvement, with a specific improvement in emotion expression, while the short allele group showed improvement in eye movement. By showing that the different genotypes are linked to different responses to SSRIs, this study implicated the genotypes in different symptom profiles.

ASD phenotypes for 5-HTTLPR genotypes. To my knowledge, only two studies have explored possible genotype-phenotype relationships for 5-HTTLPR in ASD.

Tordjman et al. (2001) attempted to correlate severity of ASD with the 5-HTTLPR alleles. They used the Autism Diagnostic Interview-Revised (ADI-R) to rate severity of social communication and interaction impairment in 71 French children with ASD. The sample of 71 children consisted of 26 with the *l/l* genotype, 35 with the *l/*s genotype, and 10 with the *s/s* genotype. Given the similar performance between the *l/s* genotype and *s/s* genotype, a possible dominant short allele effect on serotonin transport, and to preserve power when examining effects for the short allele, participants with either of these genotypes were collectively grouped in a reduced transmission group. Tordjman et al. (2001) found that total ADI-R scores varied across genotypes. Specifically, they found that the children with only long alleles (i.e. the *l/l* genotype) presented with mild to moderate impairment, while those

with a short allele (i.e. either the *s/s* or *l/s* genotype) presented with severe impairment. Tordjman and colleagues (2001) interpreted this finding as evidence for 5-HTTLPR's role in modifying the severity of deficits associated with ASD, rather than as contributing toward the risk for ASD.

The Tordjman et al. (2001) study had several limitations. They only assessed impairment in social communication and interaction, but did not assess restricted and repetitive behaviours and interests, thus neglecting a core symptom domain necessary for an ASD diagnosis. They did not report further details on their sample, such as key demographic details or information regarding DSM-IV-TR subtypes (as they conducted their study prior to the release of the DSM-5), so one is not able to establish whether their participants reflected a range of ASD presentations, or whether they recruited a sample that represented a specific or limited subgroup. Despite these limitations, it did serve to provide a foundation for future research into relationships between 5-HTTLPR and ASD characteristics.

Brune and colleagues (2006) measured behavioural characteristics in 73 children with ASD using the ADI-R and the Autism Diagnostic Observation Schedule (ADOS) across the 5-HTTLPR genotypes. Their sample consisted of 21 children with the *l/l* genotypes, 36 with the *l/s* genotype, and 16 with the *s/s* genotype. They were unable to replicate the Tordjman et al. (2001) finding that overall ADI-R scores varied across genotypes. They found the children with the short allele (i.e. the *s/s* and *l/s* genotypes) were more severely impaired on the "failure to use non-verbal communication to regulate social interaction" domain of the ADI-R, while the group of children with only long alleles (i.e. the *l/l* genotype) were more severely impaired on the "stereotyped and repetitive motor mannerisms" and aggression measures of the ADI-R, and had greater severity of impairment on directed facial expressions and unusual sensory interests on the ADOS. Brune and colleagues (2006) therefore predicted that the short allele is implicated in greater impairment in non-verbal behaviour to regulate social interaction, while the long allele is implicated in increased stereotyped and repetitive mannerisms. In terms of the core symptoms of ASD, this would implicate the *s/s* and *l/s* genotypes in social communication and interaction, and the *l/l* genotype in restricted and repetitive behaviours and interests.

Brune and colleague's (2006) results, however, must be interpreted with caution. They used subscales to try identify specific areas of impairment in ASD that could be influenced by 5-HTTLPR genotypes. They concede that their results depend on the ADI-R subscales validly measuring deficits in specific aspects of social communication and interaction, but that to date factor analysis has failed to provide consensus on its underlying structure. Further, the ADI-R

looks at past behaviour, while the ADOS assesses current behaviour, so these measures are not equivalents and cannot be treated as such.

These studies indicate that ASD phenotypes could exist for 5-HTTLPR, but they do not adequately reveal what they would be. 5-HTTLPR is clearly implicated in ASD, but whether the genotypes result in different phenotypes remains to be established.

Rationale and Aims for the Current Protocol

ASD is a very diverse disorder, but all cases are characterised by core deficits in social communication and interaction, and in restricted and repetitive behaviours and interests. The recent release of the DSM-5 resulted from a shift from a categorical understanding of ASD, with DSM-IV-TR ASD subtypes, to a dimensional understanding of the disorder. To date there is still very little knowledge regarding the underlying mechanism(s) and causes of ASD and it is unlikely that a single cause will be found for ASD. There is therefore a need to investigate possible mechanisms behind different symptom presentations.

ASD has a diverse range of presentations, but the deficits in the core symptom domains must be present in all cases. Research into the mechanisms that could underlie impairment in these core domains should be used to lay the foundation for genotype-phenotype studies. Researchers can then move toward establishing clearer phenotypes by identifying other key signs and symptoms and developing measures to assess these in a meaningful way.

A proper understanding of the fundamental aspects of ASD is necessary for any meaningful research into contributing factors. The DSM-5 was released with new ASD diagnostic criteria. This included the merging of social communication and social interaction into a single dimension. Some research suggests these two areas are fundamentally intertwined, but other researchers oppose merging these two symptom domains. Further examination of this question is therefore required.

Deficits in ToM have been proposed as underlying the social awkwardness seen in ASD, so ToM could be a mechanism behind impaired social communication and interaction. Further, the high heritability estimate of ASD has resulted in a great deal of interest in genetic contributions to the disorder. 5-HTTLPR mediates serotonergic transmission in the central nervous system and has been proposed as a candidate gene for ASD. Current studies tentatively indicate that the short allele could result in a more severe presentation that is dominated by impairment in social communication and interaction. Studies on typically

developing children, and some on individuals with ASD, have found a positive correlation between ToM and social abilities. Further, serotonin is implicated in aspects of social ability. If a genotype is linked to impaired social competence, then this genotype could be linked to impaired ToM. Currently both ToM and 5-HTTLPR are proposed as separate possible underlying mechanisms for ASD, but there is also a possibility that they are more directly linked to one another.

ASD, ToM, and 5-HTTLPR appear to be interconnected, but the nature of these relationships is unclear. This protocol aimed to directly examine these relationships. This protocol was designed to establish whether 5-HTTLPR genotypes are linked to core ASD symptoms, to establish whether ToM ability is correlated with impairment in core ASD symptoms, and to explore whether 5-HTTLPR is related to ToM ability.

This protocol is the first in a series of studies on the biological bases of social deficits in ASD. ASD scales were therefore piloted in this study, and assessment of their performance in the current sample will inform later genetic research on ASD phenotypes.

The following questions were asked:

1. How well do ASD scales used internationally to assess the core symptom domains perform in a local ASD sample?
    a. Do the scales have strong internal consistency?
    b. Does factor analysis support the given structures in regards to subscales?
2. Are core ASD symptoms better represented by the DSM-IV-TR diagnostic criteria, with three main domains of social interaction, social communication, and restricted and repetitive behaviours and interests, or by the more recent DSM-5 diagnostic criteria, which merge social communication and social interaction?
3. Is there a relationship between serotonergic transmission, as mediated by 5-HTTLPR genotypes (*l/l, l/s, s/s*), and the core ASD symptom domains of social communication and social interaction, and restricted and repetitive behaviors and interests?
4. With regards to ASD, ToM, and the 5-HTTLPR genotypes:
    a. Does ToM ability correlate with core ASD symptom impairment?
    b. Does ToM ability vary across 5-HTTLPR genotypes?

These questions, as well as the literature discussed above, led to the development of the following specific hypotheses:

1. Scales used to assess core ASD symptoms will do so adequately in a local sample as ASD is a neurodevelopmental disorder that appears consistent across race and cultures.
2. Analyses of symptom domains will support the DSM-5 diagnostic criteria over the DSM-IV-TR diagnostic criteria.
    a. Social communication and social interaction represent a single symptom domain.
    b. The symptom domain social communication and social interaction is independent of the symptom domain restricted and repetitive behaviours and interests.
3. 5-HTTLPR genotypes / alleles will relate to the level of impairment in ASD symptom domains.
    a. Children carrying the long allele will present with greater impairment in restricted and repetitive behaviours and interests than those with only short alleles.
    b. Children carrying the short allele will present with greater impairment in social communication and social interaction than children with only long alleles.
4. ToM ability will be related to core ASD symptoms, and ToM ability will relate to serotonergic neurotransmission as indicated by 5-HTTLPR genotypes.
    a. Increased ToM ability will correlate with reduced impairment in social communication and social interaction.
    b. Children carrying the short allele will present with greater impairment in ToM ability.

Method

Research Design

A relational design was used to document associations among core ASD symptoms, ToM, and 5-HTTLPR genotypes. This approach allowed each variable to be measured independently and associations between variables to emerge. This protocol consisted of preliminary analyses, Study One, and Study Two. The preliminary analyses assessed the psychometric properties of the core symptom scales and then used that data to assess whether the DSM-IV-TR or DSM-5 diagnostic criteria for ASD were better supported in this sample. Study One investigated possible relationships between core ASD symptoms and 5-HTTLPR to assess if serotonergic transmission, as mediated by 5-HTTLPR, was associated with core ASD symptoms. Study Two comprised an investigation of whether ToM was related to core ASD symptoms, and if ToM ability varied across 5-HTTLPR genotypes or alleles.

Both studies used 5-HTTLPR genotypes as a grouping factor in two ways: first, children were grouped within each study based on their genotypes (that is, the *l/l* group, the *l/s* group, and the *s/s* group); second, they were grouped on their allelic presentation to form "normal transcription" and "reduced transcription" groups, where the normal transcription group consisted of all the children with *l/l* genotypes, and the reduced transcription group consisted of those who had at least one short allele, namely, those who had the *l/s* or *s/s* genotypes. Although each genotype should have different rates of serotonergic transmission, previous studies noted that the *l/s* and *s/s* groups tended to perform similarly, thereby justifying the grouping of these two genotypes into a single "reduced transcription" group (Brune et al., 2006; Tordjman et al., 2001).

Participants

Purposive sampling was used to recruit participants. Participants were recruited from autism-specific and special needs schools in the Western Cape, as well as through the UCT Autism Research Group's database of families willing to participate in research.

DSM-IV-TR subtypes. This protocol was initiated prior to, but in anticipation of, the release of the DSM-5. At the time of recruitment, all participants held a current diagnosis of Autism, Aspergers, or PDD-NOS as specified in the DSM-IV-TR. Children with Rett's disorder were excluded as it was expected that it would be reclassified as a medical disorder, which has since occurred (American Psychiatric Association, 2013; Swedo et al., 2008). In

order to align with the release of the DSM-5, these diagnoses were used to confirm ASD status only and not as an indication of the participants' clinical presentations.

Age of participants. The age of participants was decided based on the requirements for Study Two. Children with ASD may develop ToM at different rates. Study Two needed to cater for a possible developmental delay in ToM ability, but remain sensitive to different rates of development. Participants between 7 and 14 years old were therefore recruited. Typically developing children are able to pass false-belief tasks between the ages of 3 and 5 years, but only 15-55% of children with ASD are able to pass false-belief tasks, and a significant number of these children are considerably older than 5 years of age (Happé & Frith, 1996; Ozonoff & McEvoy, 1994). A minimum recruitment age of 7 years was selected to allow to some extent for this delayed development of ToM. Further, ToM development in typically developing children has only been researched to the point of understanding social faux pas, which develops between 9 and 11 years old (Baron-Cohen et al., 1999). We therefore extended our maximum recruitment age to 14 years old to again cater for a possible delay so that older participants had a fair chance of passing social faux pas tasks. It was felt that extending the maximum age further, however, would result in an older sample where different rates of development might be masked as their ToM development would surpass the abilities measured to date in the literature as well as by the ToM measures used in Study Two.

Inclusion and exclusion criteria. Information relevant for inclusion and exclusion criteria was collected using the demographic survey (Appendix A). Children were excluded from both studies if they had a history of head injury, a history of infantile illness that could affect neurodevelopment (for instance, meningitis or encephalitis), additional co-morbid neurological disorders (for instance, cerebral palsy or epilepsy), or co-morbid psychiatric illnesses (for instance, depression). Children were also excluded if they were currently taking SSRIs as these medications directly target 5-HTTLPR, so using their genotype as a measure of the efficacy of serotonergic transmission would not be accurate. Note, however, that due to the high co-morbidity between ASD and ADHD, children with an ADHD diagnosis were not excluded. Participants whose DNA failed to genotype were excluded from Study One. All participants for Study Two were fluent in English or Afrikaans, and had to be capable of following two-stage verbal commands. Participants who met all inclusion criteria were also excluded from all studies if their parents refused consent to collect DNA, or if the child could not tolerate the cheek swab.

Final Sample. A total of 85 children with ASD were volunteered to take part in this study, of which 16 were excluded due to co-morbid neurological disorders (4 children had

Epilepsy, 2 children had Cerebral Palsy, 2 children had Fragile X Syndrome), possible brain damage (1 child had a stroke in the womb, 1 child had infantile meningitis, 1 child had unspecified structural changes noted on brain MRI), psychiatric disorders (1 child presented with selective mutism), or refusal to provide a DNA sample (3 children refused to provide DNA samples). The preliminary analyses had a final sample of 69 children. Study One included the 55 children from the preliminary analyses who were successfully genotyped. Study Two included 57 children who were able to undergo ToM testing, and their data was used to explore possible relationships between ToM and core ASD symptoms. Of the 57 children who completed ToM testing, 46 were successfully genotyped, and their data was used to explore possible relationships between ToM and 5-HTTLPR in Study Two.

Procedure

A single pool of participants was recruited for this protocol, and subgroups then progressed to further studies based on the success of DNA genotyping (for Study One), and on children's ability to complete ToM tasks (for Study Two). Parents provided informed consent for all study components before any data was collected. All data regarding core ASD symptoms were collected from parents by having them complete the symptoms scales in their own time and return these to the researcher. All data from the participants was collected on a one-to-one basis in a quiet, distraction free environment at the children's school, or at participants' home where deemed necessary. DNA was collected and the child's ability to follow two-stage verbal instructions was assessed during the first session, and, for children who continued to the second study, ToM testing was conducted over 1 to 4 sessions of between 15 and 30 minutes each.

Preliminary analyses. The preliminary analyses required data for the symptom scales. Parental consent (Appendix B) for all study components was obtained at initial recruitment. Parents completed a demographic survey (Appendix A) and symptom scales (Appendices C-E). Forms were sent home with children via schools, and returned to the researcher in the same manner. Parents received email, SMS or telephonic notifications when forms were sent home, and reminders were sent near the due dates for the return of the forms.

Study One. Study One used the data from the preliminary analyses and required DNA samples to establish each child's 5-HTTLPR genotype. The study was briefly explained to each child at the beginning of the first session, and an explanation of what was required from them was provided at the start of each subsequent session. Assent (Appendix F) was obtained at the start of the first session. In the case of non-verbal children, this assent was obtained

through the child nodding or showing a clear willingness to participate (for instance, by opening their mouth and waiting for the swab). The DNA sample was then collected using cheek swabs.

The NEPSY-II Comprehension of Instructions task (Brooks, Sherman, & Strauss, 2009) was administered during this first session to assess if the participant was suitable for Study Two. Participants who could not complete 2-stage verbal commands completed participation at this point, while those who passed progressed to Study Two.

Study Two. Study Two required participants to complete a ToM battery across several sessions to prevent fatigue. These sessions were limited to 30 minutes each, although they were often shorter depending on the child's ability to remain engaged. The ToM battery consisted of four levels, and children completed one level per session until they either completed the battery or failed a level. If children lost focus or experienced fatigue, they were given a short break or that level was split across two sessions. At the end of each session the child was thanked for their participation and provided the opportunity to ask any questions about what was done during the session or about the study. If the child was going to return for another session at a later date, the researcher explained what would happen in the next session. To prevent/reduce anxiety, children were also provided with an estimated date and time for the next appointment.

Measures

The preliminary analyses utilised a demographic survey and the scales to assess core ASD symptoms. Study One used the data from the preliminary analyses as well as genotyping data and the NEPSY-II Comprehension of Instructions Task. Study Two used all scales from Study One, as well as the UCT Autism Research Group ToM battery.

Demographic survey. Demographic data was collected for each participant (Appendix A). This included age, sex, race, socioeconomic status, and home language. Additional questions ascertained whether participants had any co-morbid neurological disorders, had a history of neurological disease, had experienced any head traumas, or had any psychiatric illnesses. This survey also established whether participants were currently using any medications.

5-HTTLPR genotypes. The DNA samples were collected by the researcher, and genotyping was conducted under the researcher's co-supervisor at the UCT Department of Molecular and Cell Biology. DNA samples were obtained from each participant by collecting buccal cells from the inside of the cheek, using a sterile swab (Buccal Epicentre© Swab).

Two to three samples were collected per participant to ensure adequate DNA collection. Once DNA was collected, it was placed in lysis buffer and transported to the UCT Department of Molecular and Cell Biology for refrigeration. Later samples were collected using a dry method, and these were stored without buffer at room temperature. DNA was then extracted with a standard extraction method using proteinase K, followed by ethanol precipitation. The DNA was re-suspended in TE buffer and stored at -80°C until genotyping analysis.

The genotypes of participants were determined using polymerase chain reaction (PCR) with oligonucleotide primers that amplify the 5-HTTLPR loci (Arieff et al., 2010). The alternate alleles were resolved by agarose gel electrophoresis and the genotypes were scored for each individual.

Core ASD symptoms. Core ASD symptoms were assessed in each participant. As discussed above, the DSM-5 was only recently released (American Psychiatric Association, 2013). The DSM-IV-TR described three core areas of impairment in ASD, namely: social communication, social interaction, and restricted and repetitive behaviours and interests (American Psychiatric Association, 2000). The new DSM-5 merged social communication and social interaction into a single dimension. This protocol was designed prior to the release of the DSM-5, and was therefore based on the DSM-IV-TR diagnostic criteria. However, the data was collected in such a way that it could be used to assess whether the DSM-IV-TR or DSM-5 approach to social communication and social interaction was more suitable.

This protocol is the first in a series of studies investigating possible biological mechanisms underlying the social deficits in ASD. One of the main aims of this protocol was to assess the suitability of ASD scales for a local ASD sample. This protocol focused on the core ASD symptoms of social communication and interaction and restricted and repetitive behaviours and interests. The scales assess a range of ASD characteristics, but as the focus was on the core ASD symptom domains, items or subscales that did not assess these would be omitted from later analyses.

The following scales obtained first-hand ratings from parents or caregivers who have long-established familiarity with the children in naturalistic social settings. These scales were not translated, but were used in their original English format to ascertain whether later studies would need translations.

Autism Social Skills Profile (Bellini & Hopf, 2007). Social competence was assessed using the Autism Social Skills Profile (ASSP) scale (Appendix C) (Bellini & Hopf, 2007). This scale was specifically developed to provide a comprehensive measure of current social functioning in individuals between the ages of 6 and 17 years who have been diagnosed with

ASD. The scale mainly focuses on social interaction, although some items do assess social communication. The ASSP includes 49 items and takes between 15 and 20 minutes to complete. The items cover a broad range of social behaviours typically exhibited by individuals with ASDs, and factor onto three subscales: social reciprocity; social participation/avoidance; and detrimental social behaviours. Items are rated on a 4-point likert scale that ranges from 'never' to 'very often'. These scores yield a final score for overall social functioning, where a higher score corresponds to greater social competence. For consistency with the other scales used in this protocol, scores were reversed so that higher scores indicated greater deficits.

The scale was initially developed to track progress for children who enter intervention programs and is therefore said to be sensitive to subtle differences in ability (Bellini & Hopf, 2007). As the current protocol recruited children who all held current ASD diagnoses, we required a scale that was sensitive to differences in impairment within an ASD sample, and thus the ASSP seemed to be suitable.

The ASSP is reported to be psychometrically sound, with a test-retest reliability of .90 and internal consistency of Cronbach's $\alpha = .93$ (Bellini & Hopf, 2007). These psychometric properties were established from a sample of 340 children with ASD between 6 and 17 years of age in the United States of America. Unfortunately, no race or SES information is provided for the sample, and as such the generalisability of these psychometric scores to other populations, such as the diverse South African population, is not known. As a relatively new scale for use in ASD research, further assessment of its validity and reliability is required.

Social Communication Questionnaire (Rutter, Bailey, & Lord, 2003).

Communication was assessed using the Social Communication Questionnaire (SCQ) (Appendix D). The SCQ mainly assesses impairment in the social communication symptom domain, although one subscale assesses social interaction and another assessed restricted and repetitive behaviours and interests. It was originally intended that the subscales not assessing social communication would provide scores to be used as collateral to support scores from the ASSP (for social interaction) and RBS-R (for restricted and repetitive behaviours and interests).

The SCQ contains 40 yes/no items and takes approximately ten minutes to complete. The SCQ has a Current and a Lifetime Form. The Current Form is for children between 2 and 5 years old, and the Lifetime Form is for use in children older than 5 years, and was therefore used in this study. The Lifetime Form focuses on the child's communication ability across their life, with a specific focus on the period between 4 and 5 years old. The scale provides a

total score and has four subscales: social interaction; communication; abnormal language; and stereotyped behaviours.

The SCQ has been shown to provide good differentiation between different levels of social communication impairment, but does mainly aim to identify the presence or absence of deficits. We anticipated that the ASSP would be more sensitive to differences in impairment in social communication and interaction, but hoped that, as the SCQ is more widely used (Eaves, Wingert, Ho, & Mickelson, 2006; Krakowiak et al., 2008; Lerner et al., 2011; Verhoeven et al., 2012b; Witwer & Lecavalier, 2007), scores on this measure would provide support for ASSP scores.

The SCQ has well-established validity and reliability (Berument, Rutter, Lord, Pickles, & Bailey, 1999; Eaves et al., 2006). Some debate exists around the cut-off scores used to indicate the presence of ASD, but this did not affect the outcome of this protocol (Eaves et al., 2006). Unfortunately, these psychometric properties have been mainly assessed in Western, predominantly English-speaking samples that may not be comparable to the diverse South African population.

Berument et al. (1999) assessed the SCQ's psychometric properties in a sample of 160 children in the United Kingdom. The sample included 147 children with ASD diagnoses, 7 with Fragile X diagnoses, 5 with Rett Syndrome diagnoses, and 40 children without any pervasive developmental disorders. They found an overall alpha reliability of .90, discriminant validity of .90, and that the overall SCQ score correlation to the ADI algorithm scores was high at 0.71. Eaves, Wingert, Ho and Mickelson (2006) assessed the psychometric properties of the SCQ in a sample of 151 children with ASD diagnoses between the ages of 3 and 7 years old. They found a sensitivity of .71 and that specificity scores ranged between .62 and .74. Both studies recruited predominantly first language English speaking children, and did not provide detailed demographic information (e.g. racial and socioeconomic status breakdown in the sample). To my knowledge, only one study has explored the psychometric properties of the SCQ in a non-westernised society. Gua et al. (2011) assessed the psychometric properties of the Chinese version of the SCQ in 736 children with ASD diagnoses between 2 and 18 years old age. They found test-retest reliability of .77-.78, internal consistency of .73-.91, and concurrent validity with the ADI-R of up to .65 in a subsample of 317 participants. Further studies need to explore the validity and reliability of the SCQ in other populations globally, especially in populations with greater racial diversity, as well as across socioeconomic strata.

Repetitive Behavior Scale – Revised (Lam, 2004). The severity of restricted and repetitive behaviours and interests was assessed using the Repetitive Behavior Scale – Revised (RBS-R) (Appendix E). The RBS-R measures the full spectrum of repetitive behaviours in ASD and consists of six subscales, namely: Stereotyped Behaviour; Self-injurious Behavior; Compulsive Behavior; Routine Behavior; Sameness Behavior; and Restricted Behavior (Gabriels et al., 2005). This scale consists of 43 items and takes approximately 15 minutes to complete. Each item is answered on a 4-point likert scale with scores ranging from 0 to 3, where higher scores indicate greater impairment. Together these items produce a single score representing the individual's level of impairment. The RBS-R was designed specifically for use within ASD samples, and includes an extensive range of behaviours, and was expected to reliably identify different levels of impairment in restricted and repetitive behaviours and interests within the current sample.

The psychometric properties of this test have been found to be within an acceptable range for clinical rating scales, with test-retest reliability of .71, inter-rater reliability of .88, and internal consistency alpha scores of between .78 and .91 (Lam & Aman, 2007; Mirenda et al., 2010). These scores were found in a sample of 307 participants from South Carolina, of which 81.40% had confirmed ASD diagnoses. The sample was predominantly Caucasian (only 23.17% African American), and had a wide age range of between 3 and 48 years. A similar study in 2010 on 287 preschool children with ASD, which was again predominantly Caucasian (71.80%), found similar results (Mirenda et al., 2010). Studies assessing the use of the RBS-R outside of the United Kingdom and the United States are lacking.

The scales used to assess core ASD symptoms in this protocol do not have reliability and validity scores for use in South African samples. However, due to the lack research on ASD locally, the protocol had to rely on scales that had established reliability and validity in other countries. It was recognised that these scales may not have been ideal for use in a sample from South Africa, but were selected as the most likely to capture useful data. This protocol intended to pilot the use of these scales to inform later phenotype studies on genetic contributors to ASD.

NEPSY-II Comprehension of Instructions *(Brooks et al., 2009).* Study Two required basic language use and it was therefore necessary to ensure that children were able to understand language and to follow verbal commands. The Comprehension of Instruction task, a subtest from the Developmental Neuropsychological Assessment, Second Edition (NEPSY-II) (Brooks et al., 2009), was administered to all verbal participants. This subtest was developed for children aged 3-12 years, but has subsequently been adapted for children aged

3-16 years. The Comprehension of Instructions subtest assesses a child's language ability by specifically testing auditory comprehension of instructions, and the ability to process and execute those instructions. The examiner issues verbal commands of increasing syntactical complexity that require the child to point to the correct picture within a series. Comprehension beyond this level was not assessed. Failure on this test indicates poor receptive language, poor linguistic or semantic knowledge, or impairment in following multi-step instructions (Brooks et al., 2009). The NEPSY-II has established psychometric properties, and the Comprehension of Instructions subtest itself has test-retest reliability ranging from .71 to .82 depending on the age of the sample. The validity of this subtest is derived from that of the NEPSY-II as a whole.

The University of Cape Town Autism Research Group Theory of Mind Battery (Hoogenhout & Malcolm-Smith, 2014). The University of Cape Town Autism Research Group Theory of Mind Battery (UCT ToM Battery) was used to assess ToM abilities (Hoogenhout & Malcolm-Smith, 2014). This battery is adapted from that of Steele et al. (2003), Baron-Cohen et al. (1999), and Happé (1994). It contains eleven tasks across four modules of increasing difficulty. Tasks include control and test questions (apart from Pretend Play and Sticker Hiding tasks). Other than the Faux Pas task, all tasks either use dolls or pictures to minimize language and memory demands. This battery provides an overall ToM score which indicates the child's ToM ability, as well as scores for each module.

Early module. The early module consisted of the Pretend Play task, the Desire task, and the Perception-Knowledge task. Pretend play develops in typically developing children at between 14 and 24 months of age (Frith & Frith, 2003), and children tend to start understanding other people's desires at two years of age (Wellman & Woolley, 1990).

The Pretend Play task tested the child's ability to use a doll as an independent agent in a pretend situation. Participants were required to complete four structured play scenarios by using the dolls to act out each event. This task was adapted to overcome a female-gender stereotype, so the stories depicted gender-neutral events (for instance, the child had to act out the doll brushing a toy dog).

The Desire task (Steele et al., 2003; Wellman & Woolley, 1990) tested the child's ability to predict a character's behaviour based on that character's desire. The task contained two picture stories that each presented a situation where a character looked for an object in one of two possible locations. The character then failed to find the object in the first location, so the examiner asked the child what they thought the character would do next, and why they would do that.

The Perception-Knowledge task (Pratt & Bryant, 1990; Steele et al., 2003) tested the child's ability to know that a character could obtain knowledge through visual access. This task consisted of four control questions and four test questions. For the test questions one doll looked into a box and another doll pushed the box without looking inside it. The child was then asked which of the dolls knew what was inside the box.

Basic module. The basic module consisted of the Location-Change False-belief task, the Unexpected-Contents False-belief task, the Explanation of Action task, and the Sticker Hiding task. Typically developing children begin to understand their own and other's false beliefs between three and five years of age (Bibby & McDonald, 2005; Naito, Komatsu, & Fuke, 1994).

The Location-Change False-belief task (Wimmer & Perner, 1983) consisted of two picture stories in which an object's location was altered while the main character was out of the room. The child's ability to understand whether this character would know where the object was, where they would look for it, and why they would look in that location was assessed.

The Unexpected-Contents False-belief task (Perner, Leekam, & Wimmer, 1987) assessed the child's ability to understand that characters have independent access to information. It made use of four familiar containers (for example, a crayon box), but where each contained unexpected contents (for example, the crayon box might contain a key). The child was asked what they initially expected to find in the container, and was then shown the unexpected content. They were then asked what a new agent who would enter the room now (meaning they would not have seen the unexpected contents) would expect to find inside the container.

The Explanation of Action task (Tager-Flusberg, personal communication, March 14, 2008) assessed the child's ability to explain the actions of others. It contained twelve stories that each portrayed an action based on an emotion, a desire, a cognitive process (i.e. thinking, knowing, or forgetting), or a non-mental event. The child was asked to explain why the character performed that action. If the child was not able to answer, they were prompted by the examiner being more specific and asking, "What is going on in [the character]'s head when s/he performed the action?"

The Sticker Hiding task (Devries, 1970) assessed the child's ability to use deception to hide a sticker from the examiner. This task was naturalistic and non-verbal, and required the child to consider the examiner's perspective and knowledge in order to keep the sticker's location a secret. Simply hiding the sticker (for instance, placing it in the left hand and closing

that hand) was not sufficient to deceive the examiner, so the child had to add an act of deception (for instance, closing the right hand so that the examiner would not know which hand held the sticker). The task started with six practice trials were the experimenter hid the sticker(s) in one, both, or neither hand so that the child's guesses were correct at least once, and incorrect at least once. The child was then given the sticker for ten trials. The last five of these trials were scored for their ability to successfully hide the sticker from the examiner. The child received points (1) for placing both hands behind their back to hide the sticker, (2) for bringing both hands to the front, (3) for keeping both hands closed until the examiner has tried to guess the sticker's location, (4) for keeping the sticker completely hidden in the hand, and (5) for using a switching strategy both for guessing and for hiding.

Intermediate module. The intermediate module consisted of the Second-Order False-belief task and the Strange Stories task. Typically developing children begin to understand second-order beliefs at between five and seven years of age (Bibby & McDonald, 2005).

The Second-Order False-belief task (Ozonoff & McEvoy, 1994) consisted of two picture stories that tested the child's knowledge regarding one character's beliefs about a second character's beliefs. The participant was asked three questions: an ignorance question (for example, "Does Mom *know* what he is making her for Mother's Day?"); a belief question (for example, "What does Mom *think* he is making her for Mother's Day?"); and a justification question (for example, "*Why* does mom think he is making that?").

The Strange Stories task (Happé, 1994) consisted of 18 illustrated stories that assess the child's ability to understand the different utterances of characters within context. These stories were subdivided into 13 types: non-mental inference (control), lie, white lie, joke, pretend, double-bluff, persuasion, forgetting, misunderstanding, figure of speech, appearance-reality, irony, and contrary emotions. The child was asked whether the character's utterance was true, and was then asked why the character had said what they had said.

Advanced module. The advanced module of this ToM battery consisted of the Lies and Jokes task and the children's version of the Faux Pas task. Typically developing children are able to differentiate between lies and jokes between six and ten years of age (Ackerman, 1981; Brüne & Brüne-Cohrs, 2006; Pollio & Pollio, 2008), and develop the ability to recognise social faux pas at between nine and eleven years of age (Baron-Cohen et al., 1999).

The Lies and Jokes task (Steele et al., 2003; Winner, Brownell, Happé, Blum, & Pincus, 1998) consisted of four picture stories that assessed the child's ability to differentiate between lying and joking. Two of the stories contained a lie, and the other two contained a joke. The stories showed a child saying something that their parent knew was untrue. In the

joke version, the child character knew that the parent was aware of the truth, and in the lie version the child did not know that the parent was aware of the truth. The participant was asked whether the child character's statement was a lie or a joke.

The children's version of the Faux Pas task (Baron-Cohen et al., 1999; Stone, Baron-Cohen, & Knight, 1998) contained five control stories that depicted a normal social event, and five test stories where a character said something awkward or embarrassing, and assessed the child's ability to differentiate between them. The child was asked, "Did anyone say something they should not have said, or something awkward?" If the child said yes, then he / she was asked, "Who said something they should not have, or something that is awkward?", "Why shouldn't he / she have said it or why was it awkward?", and "Why do you think he / she said it?"

In the original task by Baron-Cohen and colleagues (Baron-Cohen et al., 1999) ten stories were used to form ten control stories and ten test questions. A single story was therefore used twice, with only one detail being changed between the control and the test use (that is, the statement was either neutral or uncomfortable). The UCT Autism Research Group's battery only used each story once to avoid any confusion brought on by the repetition of the stories, and to prevent the child from simply repeating his or her answer from the first time they heard that story. Using the stories from the original version, this version was split into two versions such that if the test form of one story was in Version A, then its control form was in Version B, so that no stories were repeated for an individual child. The versions were alternated between participants.

Administration of the ToM battery modules. Wellman and Liu (2004) found that if children passed more advanced ToM tasks then they would pass all easier ToM tasks. In order to obtain reliable ToM scores, but still keep assessment short so that the children did not experience fatigue or lose focus, most children were started on the Basic module of the battery. If a child obtained at least half of the maximum score for this module they were awarded full points for the Early module. If they did not, they were then assessed using the Early module. However, younger children and those who were low functioning were started directly on the Early Module.

Younger children (i.e. 8 years and younger) were started on the Early module as a delay in ToM development compared to typically developing children was expected. Children considered low functioning were started on the Early module if the researcher felt they would struggle with the Basic module. This decision was made qualitatively based on the researcher's interaction with the child during DNA collection and the Comprehension of

Instruction Task (i.e. if the child performed slowly on the Comprehension of Instruction Task, if the child required additional assistance being escorted to and from the room being used for the assessment, and other indications of possible poor functioning). The children who started on the Early module and passed then progressed to the Basic Module. Children who passed the Basic module progressed onto the Intermediate module, and then the Advanced module, until they either completed the battery, or received less than 50% for a given module.

Ethical Considerations

This protocol was conducted in line with the ethical guidelines for research with human subjects as outlined by the Health Professions Council of South Africa (HPCSA) and the UCT Codes for Research. Ethical approval was obtained from both the Faculty of Science Ethics Board and the Psychology Department Ethics Board at the University of Cape Town. Permission was obtained from the Western Cape's Education Department and school principles to approach students and their families to request participation.

Participants' parents or guardians provided written informed consent before children were approached. Informed assent was obtained during the first session with each child (Appendix F). Participants and their families were assured that all data would be kept confidential, that the study posed no risk to the children, and that they could withdraw at any point without negative consequences.

All data is securely stored at the University of Cape Town's Department of Psychology, while all DNA samples are stored at the University of Cape Town's Department of Molecular and Cell Biology. The consent form utilised during recruitment (see Appendix B) required parents to provide an additional signature to consent to DNA being collected, stored, and used for later research at the University of Cape Town. Only participants whose parents provided this full consent were recruited for the study.

This protocol involved no harm to children or parents. The only risks were that some of the children might not have felt comfortable regarding the cheek swab, and might have become fatigued during testing. In order to facilitate the child feeling comfortable with swabbing, they were allowed to practice swabbing on themselves by mimicking the researcher swabbing herself. Only once they were comfortable were the DNA samples collected. If a child did not feel comfortable with the swab then they were withdrawn from the study without consequence. If the child became fatigued during testing then they were given a break or the session was split into two shorter sessions.

Feedback on completion of study. On completion of the research project, all school principles, school psychologists and participants received written feedback outlining the results of the study. This feedback also included information on the aims of the study, the methods, and an explanation of the relevance of the findings. Contact information was provided and parents and schools were encouraged to contact the researcher if they had any questions regarding the study. The researcher offered to conduct presentations at the participating schools for both teachers and parents as this would provide an interactive environment in which to address questions. These were arranged for the start of the following school year.

A written report and the thesis were sent to the Western Cape Education Department, as was stipulated as a condition when they granted permission for the study to be conducted in local schools. The thesis will be made available to the University of Cape Town's Faculty of Science Ethics Board and the Psychology Department Ethics Board.

Data Analysis

All statistical analyses were completed using the IBM SPSS Statistics, Version 20 (SPSS Inc., 2013). This protocol aimed to investigate the relationships between core ASD symptoms, ToM ability, and 5-HTTLPR. ASD symptom scores and ToM scores were continuous. 5-HTTLPR genotypes were categorical and produced three groups – *l/l*, *l/s* and *s/s*. Analysis across 5-HTTLPR alleles produced two groups, namely the "normal transcription" group (i.e. the *l/l* genotype) and the "reduced transcription" group (i.e. the *l/s* and *s/s* genotypes).

Preliminary analyses: Assessment of ASD symptom scales and support for the DSM-5 diagnostic criteria.

Assessment of ASD symptom scales in a local ASD sample. The individual questions of the ASSP, RBS-R, and SCQ-Lifetime were used as measures of core ASD symptoms. As this protocol aimed to pilot the use of these scales in a local ASD sample, the psychometric properties of these scales were assessed. The internal consistency of each scale was assessed with Cronbach's alpha, and redundant questions were removed. The validity of the given subscale structure was assessed with principal component factor analysis with varimax rotation.

As these preliminary analyses served to pilot the use of these scales in a specific sample (i.e. a South African ASD sample), principal component factor analysis was selected. This method focuses on establishing which components exist within the sample and how

variables in the given data contribute to that component (Field, 2009). The focus on parameters within the original data from the sample rather than deriving mathematical models where factors are estimated (as in other types of factor analyses) made this approach more suitable for these analyses as it limited findings to the sample of interest.

Assessment of support for the DSM-5 diagnostic criteria. As diagnostic criteria inform how we conceptualise and research ASD, the recent change in diagnostic criteria was explored. Retained questions for each scale were assessed with principal component factor analysis with varimax rotation to test whether factor loadings better reflected the DSM-IV-TR or DSM-5 diagnostic criteria (i.e. whether social communication and social interaction represent independent constructs or not, and that these are independent of the restricted and repetitive behaviours and interests symptom domain). New factors were formed based on these models. Composite scores were calculated to represent these new subscales (i.e. factors).

The use of these three scales aimed to assess severity of impairment in the core ASD symptom domains. Where scales overlapped in the symptom domains measured, it was expected that subscales or new composite scores measuring the same domain (but from different scales) would be used to support one another.

The scores from these scales were then used to assess for possible relationships between core ASD symptoms, Theory of Mind, and 5-HTTLPR. These analyses were exploratory, and as such alpha was set to .05 throughout the protocol (unless otherwise specified) (Field, 2009). This alpha level is somewhat lenient and therefore lends itself to exploratory studies.

Study One.

Core *ASD symptoms and 5-HTTLPR genotypes.* We hypothesised that core ASD symptoms would relate to rate of serotonergic transmission, as indicated by 5-HTTLPR genotype. Based on findings reported in the literature, we expected to find higher rates of impairment in restricted and repetitive behaviours and interest associated with the long allele, and an association between greater impairment in social communication and interaction and the short allele (Brune et al., 2006; Tordjman et al., 2001). A multivariate analysis of variance (MANOVA) was conducted to establish if core ASD symptoms varied across 5-HTTLPR genotypes.

Core ASD symptoms were compared across verbal and non-verbal participants. This was done descriptively due to the very small size of the non-verbal group. Core ASD symptoms across genotypes within the non-verbal group were also examined descriptively.

Study Two. Study Two used data from the preliminary analyses and Study One, with the addition of ToM scores. ToM scores were assessed for normality to inform which statistical analyses would be conducted to test hypotheses.

Core ASD symptoms and Theory of Mind. Correlation analysis was run between ToM scores and core ASD symptoms using Spearmann's rho to assess for possible associations. We hypothesised that greater ToM ability would correlate with less impairment in social communication and interaction. One way analyses was therefore conducted, with alpha of .05 as this was again an exploratory analysis.

ToM and age across 5-HTTLPR genotypes. We hypothesised that ToM ability would be greater for children only carrying long alleles (i.e. with normal serotonergic transmission), as the literature has found the short allele is associated with greater impairment in social communication and interaction in ASD samples, and ToM ability correlates with social competence in typically developing children (Bosacki & Wilde Astington, 2001; Brune et al., 2006; Repacholi & Slaughter, 2003; Tordjman et al., 2001).

The expected correlation between ToM and age for all participants was confirmed. ToM across age for all participants was plotted to assess for a developmental pattern.

ToM scores were plotted against age for each genotype to establish whether certain genotypes exhibited a later start in ToM development, slower ToM development, or an early plateau in ToM development.

It was theorised that genotypes would predispose individuals to different patterns of ToM development, and that age would then influence how far a child was in this development. Hierarchical multiple regression assessed the collective influence of age and genotype for ToM.

ToM and age across 5-HTTLPR alleles. ToM was again assessed with hierarchical multiple regression, but where participants were divided into normal and reduced transcription groups according to alleles.

Results

Sample Characteristics

Participants. A total of 85 children with ASD were recruited. Of these, 15 were excluded due to: co-morbid epilepsy (four children); a diagnosis of Fragile X Syndrome (two children); co-morbid Cerebral Palsy (two children); brain damage (two children); selective mutism (one child); a history of meningitis (one child); and refusal to provide a DNA sample

(3 children). A further child was excluded as his parents did not provide information regarding his symptoms and his DNA sample failed to provide genotyping data, so no data was available for him.

Table 1.
Demographic Information for preliminary analyses, Study One and Study Two

	Preliminary Analyses (%)	Study One (%)	Study Two (%)
N	69 (100)	55 (100)	57 (100)
Sex			
Male	63 (91.30)	50 (90.91)	51 (89.47)
Female	6 (8.70)	5 (9.09)	6 (10.53)
Age			
Mean	9.58	9.73	9.67
SD	2.11	2.16	2.25
Range (yrs)	7-14	7-14	7-14
Home Language			
English	58 (84.06)	47 (85.45)	52 (91.23)
Afrikaans	6 (8.70)	5 (9.09)	3 (5.26)
isiXhosa	2 (2.90)	1 (1.82)	0 (0.00)
Other	3 (4.35)	2 (3.64)	2 (3.51)
Socioeconomic Status			
Low	22 (31.88)	17 (30.91)	19 (33.33)
Medium	23 (33.33)	21 (38.18)	20 (35.09)
High	22 (31.88)	15 (27.27)	16 (28.07)
Undisclosed	2 (2.90)	2 (3.64)	2 (3.51)
Race			
White	26 (37.68)	22 (40.00)	23 (40.35)
Black	7 (10.14)	5 (9.09)	4 (7.02)
Coloured	26 (37.68)	19 (34.55)	23 (40.35)
Indian	5 (7.25)	5 (9.09)	3 (5.26)
Other	5 (7.25)	4 (7.27)	4 (7.02)
Genotype	n/a		
l/l Genotype		18 (32.73)	16 (34.78)
l/s Genotype		23 (41.82)	16 (34.78)
s/s Genotype		14 (25.45)	14 (30.43)
Total		55 (100.00)	46 (100.00)

A total final sample of 69 ASD children was therefore obtained. All children were included in the preliminary analyses of the ASD symptom scales. All participants provided DNA samples for genotyping, but only 55 samples were successfully genotyped; these children were included in Study One. Of the total 69 children, 12 could not correctly complete the two-stage verbal instruction commands on the NEPSY-II Comprehension of Instructions task. Study Two therefore included 57 children from the preliminary analyses who were able to undergo ToM testing. Of these 57 children, 46 were successfully genotyped.

Data was incomplete for two participants as both were missing scores for the SCQ-Lifetime Form and ASSP, and one was missing scores for the RBS-R. However, as data was available for other measures, these children were only excluded from the analyses for which they were missing data.

Representing the ASD spectrum. This study aimed to recruit a sample of children who reflected the spectrum of ASD. Thus verbal and non-verbal children were included. As the study was designed to align with DSM-5 diagnostic criteria, we did not make use of DSM-IV-TR ASD subtypes in recruitment or analyses. However, these subtypes were noted.

The distribution of DSM-IV-TR subtypes for the total sample is shown in Figure 1. The majority of participants were diagnosed with Autism (56.52%), although we did not distinguish between high functioning and low functioning Autism. There were equal numbers of Aspergers (21.74%) and PDD-NOS (21.74%) diagnoses.

The distribution of verbal and non-verbal children across DSM-IV-TR ASD subtypes for the total sample is shown in Figure 2. Study Two excluded the 12 non-verbal children, so distribution of the verbal children across subtypes indicates the distribution of these subtypes in Study Two.

The distribution of DSM-IV-TR subtypes across 5-HTTLPR genotypes is shown in Figure 3. Subtypes were similarly distributed across genotypes, although the *s/s* genotype showed an increased portion of PDD-NOS diagnoses. No subtypes aligned with specific genotypes.

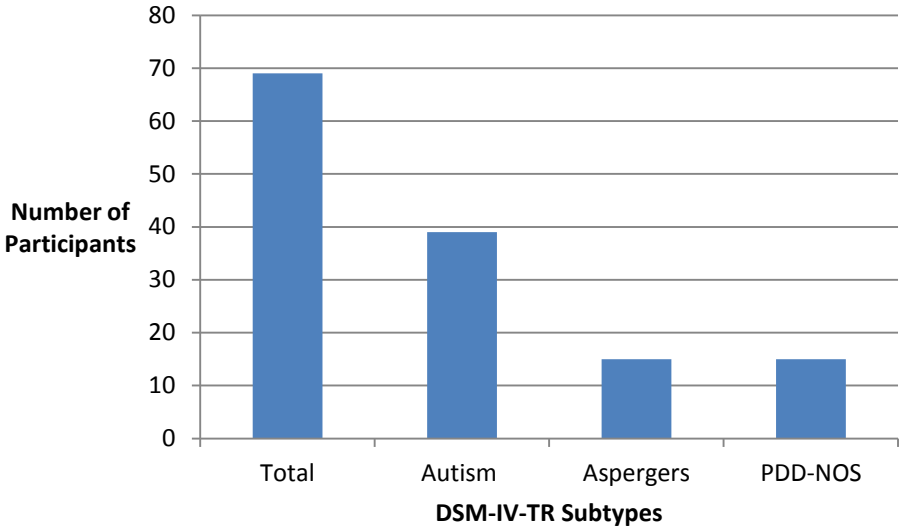

Figure 1. Distribution of DSM-IV-TR ASD Subtypes in the Total Sample

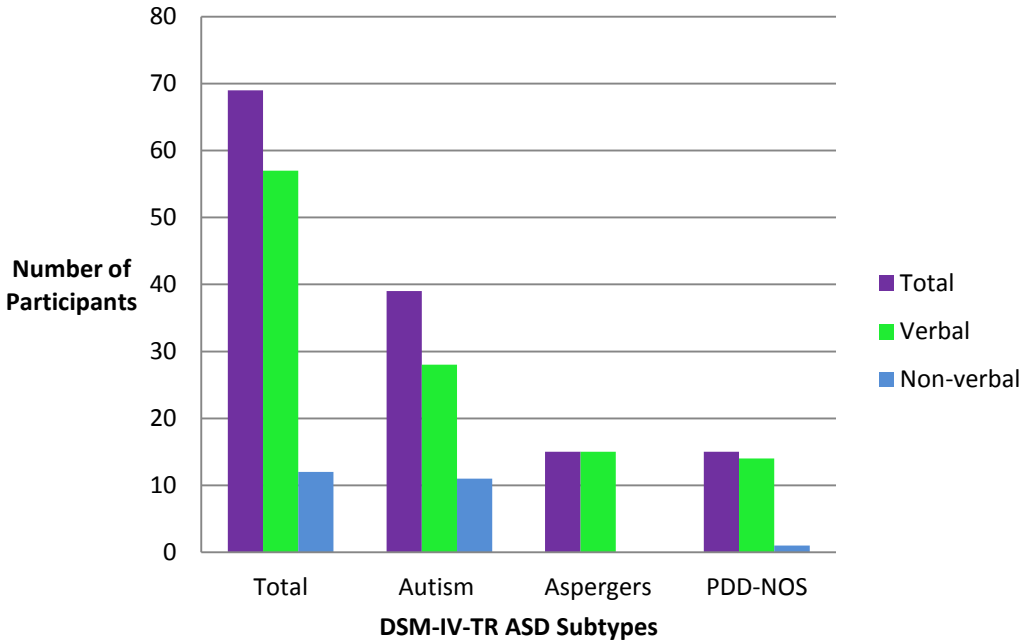

Figure 2. Distribution of Verbal and Non-verbal Participants Across DSM-IV-TR ASD Subtypes for the Total Sample

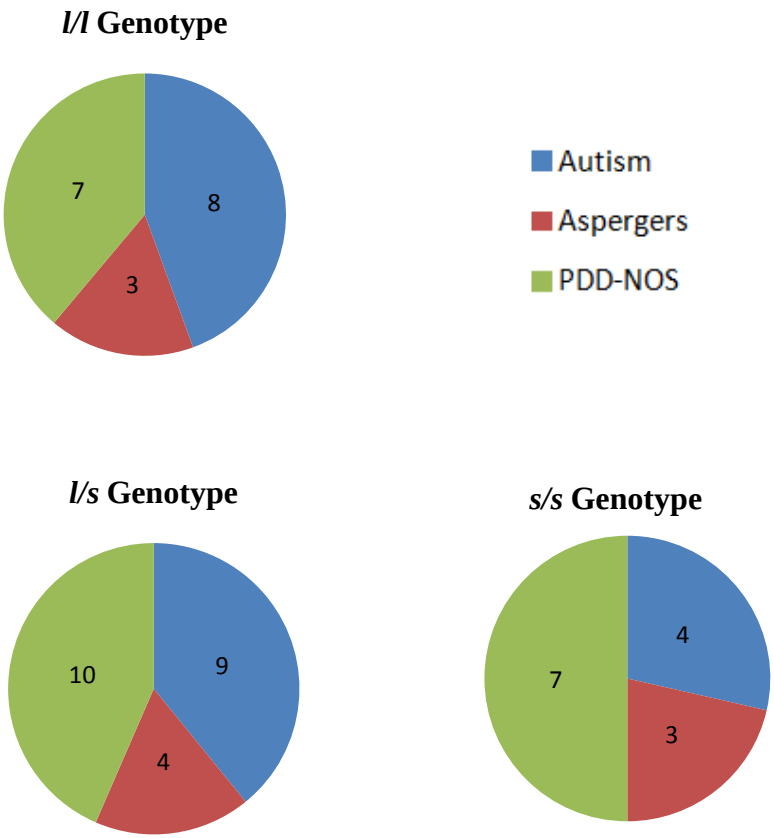

Figure 3. Distribution of DSM-IV-TR Subtypes Across 5-HTTLPR Genotypes

The distributions of DSM-IV-TR ASD subtypes in each genotype for Study Two, which only included verbal participants, are shown in Figure 4. For this sample the *l/s* genotype had a higher portion of PDD-NOS diagnoses and less Autism diagnoses than the other genotypes.

The sample in this protocol thus reflects the ASD spectrum as non-verbal and verbal children are included, and DSM-IV-TR ASD subtypes are represented in all studies.

Sex. The total sample (i.e. the preliminary analyses) was predominantly male (91.30%), with 6 female and 63 male participants (female to male ratio of 1:10.5). Study One included 5 female and 50 male participants (female to male ratio of 1:10). Study Two included 6 female and 51 male participants (female to male ratio of 1:8.5).

Age. Children between 7 and 14 years of age were recruited for this study. The mean age for each of the studies were similar: preliminary analyses, $M = 9.58$, $SD = 2.11$; Study One $M = 9.73$, $SD = 2.16$; and Study Two $M = 9.67$, $SD = 2.25$. One-way ANOVA found no between group differences in age across genotypes for Study One, $F\ (2,54) = 0.64$, $p = .53$, $r = .15$, or for Study Two, $F\ (2,43) = 0.57$, $p = .57$, $r = .16$.

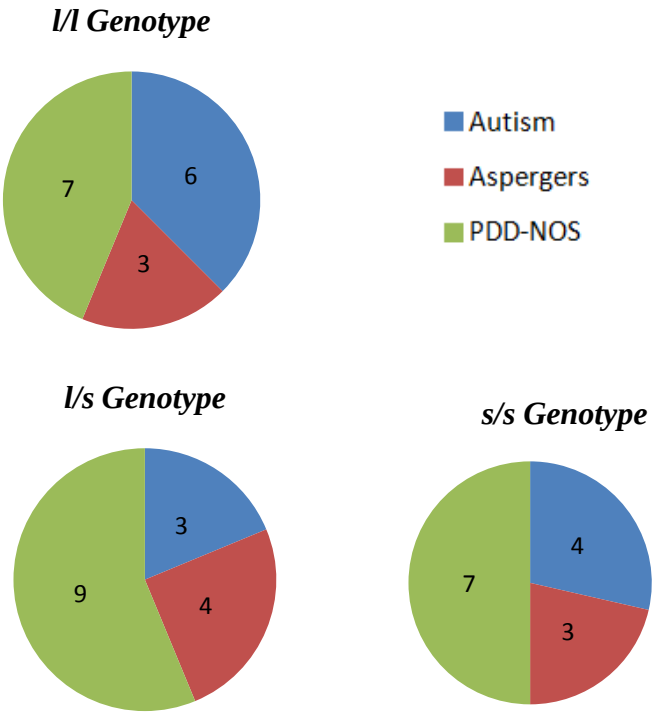

Figure 4. Distribution of DSM-IV-TR Subtypes Across 5-HTTLPR Genotypes for Study Two

Home Language. This study recruited children who were proficient in English or Afrikaans. The distribution of language across all samples is shown in Table 3. The sample mainly consisted of first language English speakers. Three children were from homes where Zulu, French, or German were primarily spoken, and were collectively categorised under "Other languages". Chi-square analyses found no significant between group differences in home language across genotypes for Study One, $\chi 2$ (6) = 7.43, p = 0.28, or Study Two, $\chi 2$ (4) = 4.42, p = 0.35, although these results must be taken with caution as some cells contained less than 5 participants.

Table 2.
Distribution of Home Language for preliminary analyses, Study One and Study Two

	Preliminary Analyses (%)	Study One (%)	Study Two (%)
N	69 (100)	55 (100)	57 (100)
English	58 (84.06)	47 (85.45)	52 (91.23)
Afrikaans	6 (8.70)	5 (9.09)	3 (5.26)
isiXhosa	2 (2.90)	1 (1.82)	0 (0.00)
Other	3 (4.35)	2 (3.64)	2 (3.51)

Socioeconomic status. Traditional markers of socioeconomic status (SES) may not be appropriate in a local context as South Africa has a substantial informal economy and resource sharing remains common (Myer, Ehrlich, & Susser, 2004). Due to this, SES was measured using the method described in Myer, Stein, Grimsrud, Seedat, and Williams (2011) which uses estimated household income, highest level of education for each parent, and an asset index. The asset index was based on 17 items deemed to reflect individual and household wealth, and included questions regarding ownership of appliances, access to resources, and the financial activities of the participants' parents or guardians (see Appendix A). Household income, parental education, and assets were then used to rank participants as having low, middle, or high socioeconomic status. This measure is designed for increased sensitivity in low SES contexts, and therefore those who rank in the high SES stratum with this algorithm may rank as moderate to high SES on traditional SES ranking systems.

The SES distribution across different sub-samples in this study is shown in Table 3. Chi-square analysis found no significant group differences in SES across genotypes for Study One, $\chi 2\ (6) = 7.63$, $p = 0.28$, or Study Two, $\chi 2\ (6) = 9.56$, $p = 0.15$, although these results must be taken with caution as some cells contained less than 5 participants.

Table 3.
Distribution of SES Across Samples in the Current Protocol

	N	*Low SES* (%)	*Middle SES* (%)	*High SES* (%)	*Not disclosed* (%)
Preliminary Analyses	69	22 (31.88)	23 (33.33)	22 (31.88)	2 (2.90)
Study One	55	18 (32.73)	23 (41.82)	12 (21.82)	2 (3.64)
Study Two	57	19 (33.33)	20 (35.09)	16 (28.07)	2 (3.51)

5-HTTLPR Genotypes. Of the 69 participants, 55 DNA samples provided genotypes. Study One recruited all participants from the preliminary analyses who were successfully genotyped (i.e. n = 55). Study Two recruited all participants who were able to undergo ToM testing (i.e. n= 57), and this included 46 children who were successfully genotyped. The genotype distributions for Study One and Study Two are shown in Table 4. We note that Study Two, which excluded non-verbal participants, had the same number of *s/s* genotypes as Study One, but a decrease in the number of those containing long alleles (i.e. the *l/l* genotype and *l/s* genotype). As the *s/s* genotype has the most reduced transcription, and this was

expected to relate to greater deficits in social communication and interaction, it was surprising that none of those children were non-verbal.

Table 4.
Genotype Distribution for Study One and Study Two

	Study One (%)	Study Two (%)
N	55 (100)	46 (100)
Genotype		
l/l Genotype	18 (32.73)	16 (34.78)
l/s Genotype	23 (41.82)	16 (34.78)
s/s Genotype	14 (25.45)	14 (30.43)

To my knowledge, only two studies have explored the distribution of 5-HTTLPR genotypes locally: one in an ASD sample (Arieff et al., 2010), and one in a sample taken from the general population (Esau et al., 2008). The distribution of genotypes across those samples and the current sample are shown in Table 5. 5-HTTLPR distribution across the current study and these two studies are discussed later.

Table 5.
Distribution of 5-HTTLPR Genotypes Across South African Samples

	N	*l/l* Genotype (%)	*l/s* Genotype (%)	*s/s* Genotype (%)
Current Study	55	18 (32.73)	23 (41.82)	14 (25.45)
South African ASD Population (Arieff et al., 2010)	109	56 (51.38)	20 (18.35)	33 (30.28)
General South African Population (Esau et al., 2008)	342	210 (61.40)	116 (33.92)	16 (4.68)

Race. The racial distribution of our sample is shown in Table 6. Our sample had increased rates of white participants and decreased rates of black participants compared to the racial demographics of the Western Cape. Race can be indicative of genetic heritage, and must always be considered in genetic studies. The racial distribution of the current study in relation to other studies on 5-HTTLPR in ASD samples is discussed later.

Table 6.

Distribution of Race Across Samples in the Current Protocol

	Preliminary Analyses (%)	Study One (%)	Study Two (%)	Western Cape (Statistics South Africa, 2012)
N	69 (100)	55 (100)	57 (100)	na
White	26 (37.68)	22 (40.00)	23 (40.35)	n/a (15.7)
Black	7 (10.14)	5 (9.09)	4 (7.02)	n/a (32.8)
Coloured	26 (37.68)	19 (34.55)	23 (40.35)	n/a (48.8)
Indian	5 (7.25)	5 (9.09)	3 (5.26)	n/a (1.0)
Other	5 (7.25)	4 (7.27)	4 (7.02)	n/a (1.7)

The characteristics of the sample recruited for this protocol were explored above and are discussed further in the Discussion section. This protocol strove to recruit a sample that reflected the ASD spectrum. Study Two could not include non-verbal children as they could not complete ToM testing, but all other analyses included non-verbal children, and all analyses included children from all DSM-IV-TR ASD subtypes. The demographic analyses indicated that the sample was diverse and did not represent a specific subgroup within ASD.

Preliminary Analyses: initial assessment of ASD symptom scales and support for the DSM-5 diagnostic criteria.

The ASSP, SCQ, and RBS-R were used as measures of core ASD symptoms. As none of these scales have reported validity or reliability statistics for use in South Africa, the psychometric properties had to be established for use in the current sample, with a view to assessing their usefulness for future work. The internal consistency of each scale was assessed with Cronbach's alpha and redundant questions were removed. Principal component factor analysis with varimax rotation assessed the latent structure of each scale to assess the validity of the given subscales. The reliability of factor analysis is dependent on sample size and ideally one would recruit 10-15 participants per variable (Field, 2009). However, due to the time and cost limitations of this being a Masters thesis, as well as the challenges conducting genetic research within an ASD sample, it was not possible to recruit such a large sample. The findings from all principal component analyses should therefore be interpreted with caution.

Support for the DSM-5 diagnostic criteria was also assessed using principal component factor analyses on each scale. These analyses compared models that aligned with DSM-IV-TR diagnostic criteria (i.e. where social communication and social interaction were separate symptom domains) and models that aligned with the DSM-5 diagnostic criteria (i.e. where social communication and social interaction are merged to form one symptom domain).

New factors were formed based on the strongest model for each scale. Composite scores were calculated to represent these new subscales (i.e. factors). The use of these three scales aimed to assess variability in core ASD symptoms in this sample.

Despite the literature reporting acceptable reliability and validity statistics for all three scales, and the fact that ASD presents similarly across various contexts, none of the scales performed well within our sample. The extent to which the scales were problematic was not anticipated, and became apparent during data analyses. In an attempt to overcome the weaknesses of these scales, the factor composite scores were used for all analyses on core ASD symptoms. Although this could not entirely overcome the shortcomings of the measures, it did protect the integrity of the data to some extent. Detailed descriptions of the assessment of each scale are discussed below.

Autism Social Skills Profile (Bellini & Hopf, 2007). The Autism Social Skills Profile was used to measure ASD characteristics and mainly focused on children's social skills, with lower scores indicating a greater deficit. As the SCQ-Lifetime and RBS-R both indicate greater deficit with higher scores, the scores of the ASSP were reversed to do the same.

Each question was answered on a likert scale from 1 to 4, where 4 points indicated the highest level of impairment. 68 children had complete data for the ASSP.

The internal consistency of the ASSP was assessed using Cronbach's alpha. Analysis found the following scores: Total Score (Cronbach's α =.60), Social Participation / Avoidance subscale (Cronbach's α =.71), Social Reciprocity (Cronbach's α =.41), and Detrimental Social Behaviour (Cronbach's α =.27). These results indicated poor internal consistency and a problem with two of the standard subscales. Individual questions were then assessed so weak items could be identified and eliminated. Items with weak correlation to the Total Score's Cronbach's alpha (that is, less than r = .30) were removed (Field, 2009). Of the 49 questions in the ASSP, 12 were identified as weak and were removed (Appendix G).

Principal component factor analysis with varimax rotation was run on the remaining 37 questions. The Kaiser-Meyer-Olkin measure verified that the sample size was adequate for factor analysis on this data, KMO = .73 (Field, 2009). However, as previously mentioned, a sample size that allowed 5-10 participants per variable would have been optimal, so thse

results must be interpreted with caution. Bartlett's Test of Sphericity indicated sufficiently large correlations between questions for principal component factor analysis, χ^2 (666) = 1539.14, $p < .001$. Initial analysis indicated 9 components with eigenvalues over Kaiser's criterion of 1, and together explained 70.35% of the total variance. The scree plot was ambiguous, and indicated points of inflection that justified retaining 2 or 3 components, which explained 27.58% and 39.67% of the total variance respectively. The scree plot is shown in Figure 5.

As the ASSP has three subscales and the scree plot suggested a three factor model, a forced three factor model was run. These loadings were compared to those of the given subscales, and it was noted that the items did not load in the same manner. Appendix H shows the factors each question loaded onto, as well as which ASSP subscale it was meant to load to. Due to this and the poor internal consistency, the ASSP subscales were not considered reliable in this sample.

Models were then assessed based on the theoretical understanding of ASD according to symptom dimensions outlined in the DSM-IV-TR and DSM-5. These models are discussed below.

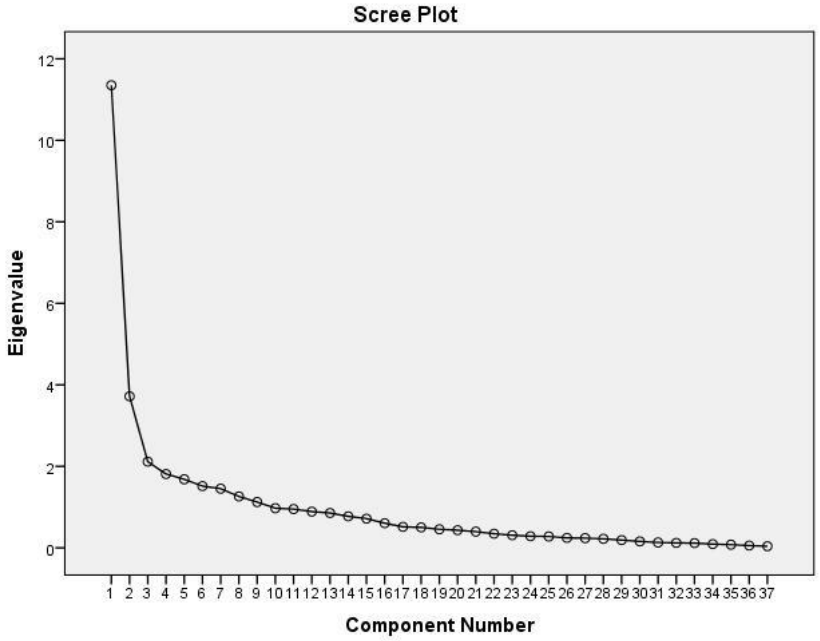

Figure 5. Scree plot based on principal component analysis with varimax rotation for the ASSP.

The ASSP mainly assesses social interaction, but does include items that assess social communication. The ASSP does not include items assessing restricted and repetitive behaviours and interest. Principal component factor analysis was used to assess whether the latent structure best supported a two factor model measuring social communication and social interaction separately (as in the DSM-IV-TR) or a single factor model merging them (as in the DSM-5).

Principal component factor analysis with varimax rotation was run forcing a 2 factor solution. This solution explained 40.73% of the total variance. Loadings were assessed to see if items clearly loaded onto two factors, namely "social communication" and "social interaction" from the DSM-IV-TR. The factor loadings for this model are shown in Table 7. Factor one appeared to represent social interaction and factor two appeared to represent social communication. However, factor two also included items that represented social interaction (for instance, "considers multiple viewpoints" and "offers assistance to others"). Several items did not load strongly onto either factor, such as "recognises the nonverbal cues or body language of others" and "maintains an appropriate distance when interacting with others". Some factors loaded approximately equally onto both factors, such as "speaks with an appropriate volume in conversation" and "joins a conversation with two or more people without interrupting".

Table 7.

Factor loadings and communalities based on principal component analysis with varimax rotation for the ASSP for a forced two factor solution after weak items were removed

	Factor 1	Factor 2	Communality
1. Invites peers to join him/her in activities	**.63**	.36	.53
2. Joins activities with peers	**.71**		.55
3. Takes turns during games and activities	**.51**	.25	.32
4. Maintains person hygiene		.36	.18
5. Interacts with peers during unstructured activities	**.76**		.58
6. Interacts with peers during structured activities	**.70**		.51
9. Engages in one-on-one social interactions with peers	**.66**	.28	.52

10. Interacts with groups of peers	**.65**	.31	.51
11. Maintains the "give-and-take" of conversations		**.56**	.37
12. Expresses sympathy for others		**.71**	.56
13. Talks about or acknowledges the interests of others		**.63**	.43
14. Recognises the facial expressions of others		.48	.25
15. Recognises the nonverbal cues or body language of others	.26	.32	.17
17. Understands the jokes or humour of others	.41	.44	.28
18. Maintains eye contact during conversations	.33		.12
19. Maintains an appropriate distance when interacting with peers			.07
20. Speaks with an appropriate volume in conversations	.44	.43	.38
21. Considers multiple viewpoints		**.55**	.36
22. Offers assistance to others		**.70**	.54
23. Verbally expresses how he/she is feeling		**.64**	.41
24. Responds to the greetings of others		**.58**	.34
25. Joins a conversation with two or more people without interrupting	.39	.35	.27
26. Initiates greetings with others		**.72**	.52
27. Provides compliments to others		**.72**	.52
28. Introduces self to others		**.79**	.62
29. Politely asks others to move out of his/her way		**.65**	.45
30. Acknowledges the compliments directed at him/her in activities		.37	.17
31. Allows peers to join him/her in activities	**.61**	.28	.45
32. Responds to the invitations of peers to join them in activities	**.75**		.60
34. Responds to questions directed at him/her by others	.40	.31	.26
35. Experiences positive peer interactions	**.60**	.25	.43

36. Compromises during disagreements with others	**.61**	.42
41. Engages in solitary interests and hobbies	**.59**	.37
44. Exhibits fear or anxiety regarding social interactions	**.70**	.50
46. Engages in socially inappropriate behaviour	**.70**	.49
47. Exhibits poor timing with his/her social initiations	**.68**	.46
49. Engages in solitary activity in the presence of others	**.74**	.57

Note: Factor loadings below .25 are suppressed

Principal component factor analysis forcing a one factor solution found that a single factor explained 30.69% of the total variance. 26 questions loaded moderately to strongly (i.e. factor loadings above .50) onto this factor. The factor loadings for this model are shown in Table 8. All questions on the ASSP assess social communication or social interaction, so the theoretical justification for scores here representing the symptom dimension of Social Communication and Interaction (as per the DSM-5) is strong.

Table 8.

Factor loadings and communalities based on principal component analysis with varimax rotation for the ASSP for a forced one factor solution after weak questions were removed

	Factor 1: Social Communication and Interaction (ASSP)	Communality
1. Invites peers to join him/her in activities	**.71**	.51
2. Joins activities with peers	**.69**	.48
3. Takes turns during games and activities	**.55**	.31
4. Maintains person hygiene	.40	
5. Interacts with peers during unstructured activities	**.65**	.42
6. Interacts with peers during structured activities	**.62**	.38
9. Engages in one-on-one social interactions with peers	**.69**	.48

10. Interacts with groups of peers	**.70**	.49
11. Maintains the "give-and-take" of conversations	**.54**	.29
12. Expresses sympathy of others	**.63**	.40
13. Talks about or acknowledges the interests of others	**.54**	.29
14. Recognises the facial expressions of others	.41	
15. Recognises the nonverbal cues or body language of others	.41	
17. Understands the jokes or humour of others	**.53**	.28
18. Maintains eye contact during conversations	.30	
19. Maintains an appropriate distance when interacting with peers	.26	
20. Speaks with an appropriate volume in conversations	**.61**	.38
21. Considers multiple viewpoints	**.53**	.28
22. Offers assistance to others	**.62**	.39
23. Verbally expresses how he/she is feeling	.46	
24. Responds to the greetings of others	.38	
25. Joins a conversation with two or more people without interrupting	**.52**	.27
26. Initiates greetings with others	**.51**	.26
27. Provides compliments to others	**.50**	.25
28. Introduces self to others	.47	
29. Politely asks others to move out of his/her way	**.53**	.28
30. Acknowledges the compliments directed at him/her in activities	.36	.13
31. Allows peers to join him/her in activities	**.65**	.42
32. Responds to the invitations of peers to join them in activities	**.71**	.51
34. Responds to questions directed at him/her by others	**.51**	.26
35. Experiences positive peer interactions	**.63**	.39

36. Compromises during disagreements with others	**.61**	.37
41. Engages in solitary interests and hobbies	**.55**	.30
44. Exhibits fear or anxiety regarding social interactions	.48	
46. Engages in socially inappropriate behaviour	**.61**	.37
47. Exhibits poor timing with his/her social initiations	.49	
49. Engages in solitary activity in the presence of others	**.68**	.47

Note: Factor loadings below .25 are suppressed

The two factor model had an unclear structure, while the single factor model aligned with the current conceptualisation of ASD symptom domains. The single factor model for the ASSP was therefore selected. Scores for all retained items (i.e. items with factor loadings above .50) were averaged and formed a "Social Skills and Communication (ASSP)" score for each participant.

Social Communication Questionnaire – Lifetime Form (Witwer & Lecavalier, 2007). The Social Communication Questionnaire – Lifetime Form mainly assessed the social communication symptom domain, although it included items for social interaction and restricted and repetitive behaviours and interests. It was initially expected that scores from the Communication and Abnormal Language subscales could be used to indicate social communication ability, while the Social Interaction subscale scores would be used to support ASSP scores and Stereotyped Behaviours subscale scores would be used to support RBS-R scores. 68 children had complete data for the SCQ-Lifetime Form.

The internal consistency of the SCQ-Lifetime Form was assessed using Cronbach's alpha. Analysis of the subscales found the following scores: Total Score (Cronbach's α =.61), Communication (Cronbach's α =.62), Social Interaction (Cronbach's α =.54), Stereotyped Behaviours (Cronbach's α =.51), and Abnormal Language (Cronbach's α =.33). As this indicated poor internal consistency, Cronbach's alpha was used to identify weak questions for removal. Items with weak correlation to the overall Cronbach's alpha (that is, less than r = .30) were removed. Of the 40 questions in the SCQ-Lifetime Form, 14 questions were removed (Appendix I).

Principal component factor analysis with varimax rotation was run on the 26 questions that were retained. The Kaiser-Meyer-Olkin measure verified that the sample size was

adequate for factor analysis on this data, KMO = .66 (Field, 2009). As the sample was smaller than the generally accepted standard of 10-15 participants per variable, however, all factor analysis results must be interpreted with caution. Bartlett's Test of Sphericity indicated sufficiently large correlations between items for principal component factor analysis, χ^2 (325) = 676.69, $p < .001$. Initial analysis indicated 8 components with eigenvalues over Kaiser's criterion of 1, and together explained 67.51% of the variance. The scree plot indicated points of inflection at 2, 3, or 4 components, where 2 components explained 19.91% of the total variance, 3 components explained 29.08% of the total variance, and 4 components explained 37.92% of the total variance. The scree plot is shown in Figure 6.

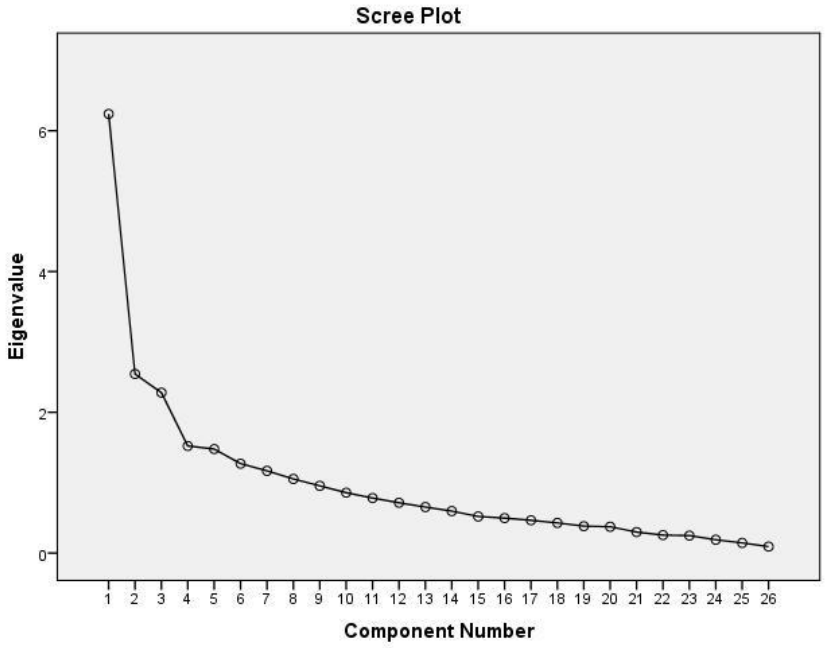

Figure 6. Scree plot based on principal component analysis with varimax rotation for the SCQ-Lifetime after weak items were removed

As the SCQ has four subscales and the scree plot suggested a four factor model, a forced four factor model was run. These loadings were compared to those of the given subscales, and it was noted that the items did not load in the same manner. Appendix J shows the factors each question loaded onto, as well as which SCQ subscale the item was meant to load to. Due to this and the poor internal consistency, the SCQ subscales were not considered reliable in this sample.

The SCQ-Lifetime contains questions that assess social interaction, social communication, and restricted and repetitive behaviours and interests. A single factor model

was therefore not possible as it did not have a theoretical justification. Models were therefore assessed to see whether the latent structure better suited the three symptom dimensions model of the DSM-IV-TR or the two symptom dimensions model of the DSM-5.

Principal component factor analysis with varimax rotation was run forcing a 3 factor solution. This solution explained 42.55% of the total variance. Loadings were assessed to see if items clearly loaded onto three separate factors, namely "social communication", "social interaction" and "restricted and repetitive behaviours and interests". Factor loadings for this model are shown in Appendix K. This model was problematic, as the first factor included items that measured social communication and interaction, the second factor included items that measured social skills and restricted and repetitive behaviours, and the third factor included items that measured social communication and interaction. This splitting of questions measuring the same ASD symptom dimension and the loading of questions measuring different ASD symptom dimensions onto the same factor was problematic.

Principal component factor analysis with varimax rotation was also run forcing a 2 factor solution. This solution explained 33.78% of the total variance. Loadings were assessed to see if items clearly loaded onto two factors, namely "social communication and interaction" and "restricted and repetitive behaviours and interests". Factor loadings for this model are shown in Appendix L. The first factor appeared to mainly represent social interaction with some social communication, while the second factor seemed to represent both social communication and restricted and repetitive behaviours and interests. The splitting of communication items across both factors is problematic. In addition, several items did not load strongly onto either factor.

As neither of the above models had a clear structure, all questions relating to restricted and repetitive behaviours and interests were removed and principal component factor analysis was run on the remaining 19 questions to compare two factor (separating social communication and social interaction) and one factor (merging social communication and interaction) models. The two factor solution is shown in Table 9. Several items loaded equally onto both factors, such as "when she/he was 4 to 5, did she/he smile back if someone smiled at her/him?" and "when she/he was 4 to 5, did she/he ever show you things that interested him/her to engage your attention?". Further, the two factor model showed arbitrary splitting of questions that should load onto the same factor, such as "when she/he was 4 to 5, did she/he show a normal range of facial expressions?" (Factor 1) and "has his/her facial expression usually seemed appropriate to the particular situation, as far as you could tell?" (Factor 2).

Table 9.

Factor loadings and communalities based on principal component analysis with varimax rotation for the SCQ-Lifetime for a forced two factor solution after removing all items not relating to social communication and interaction

	Factor 1	Factor 2	Communality
9. Has her/his facial expression usually seemed appropriate to the particular situation, as far as you could tell?		**.54**	.29
10. Has she/he ever used your hand like a tool or as if it were a part of her/his own body (e.g. pointing with your finger or putting your hand on a doorknob to get you to open the door)?	.42		.20
20. When she/he was 4 to 5, did she/he ever talk with you just to be friendly (rather than to get something)?	.46	.41	.38
22. When she/he was 4 to 5, did she/he ever spontaneously point at things around her/him just to show you things (not because she/he wanted them)?		**.56**	.34
23. When she/he was 4 to 5, did she/he ever use gestures, other than pointing or pulling your hand, to let you know what she/he wanted?		**.52**	.28
24. When she/he was 4 to 5, did she/he nod her/his head to mean *yes*?		**.79**	.63
25. When she/he was 4 to 5, did she/he shake her/his head to mean *no*?		**.79**	.63
26. When she/he was 4 to 5, did she/he usually look at you directly in the face when doing things with you or talking with you?	.35	.41	.29
27. When she/he was 4 to 5, did she/he smile back if someone smiled at her/him?	.46	.44	.40
28. When she/he was 4 to 5, did she/he ever show you things that interested him/her to engage your attention?	.33	.33	.21
29. When she/he was 4 to 5, did she/he ever offer to share things other than food with you?	.46	.33	.32
30. When she/he was 4 to 5, did she/he ever seem to want you to join in her/his enjoyment of something?	.45	.38	.35
31. When she/he was 4 to 5, did she/he ever try to comfort you if you were sad or hurt?	**.55**		.35

33. When she/he was 4 to 5, did she/he show a normal range of facial expressions?	**.54**	.36	.42
36. When she/he was 4 to 5, did she/he seem interested in other children of approximately the same age whom she/he did not know?	**.64**		.42
37. When she/he was 4 to 5, did she/he respond positively when another child approached her/him?	**.81**		.65
38. When she/he was 4 to 5, if you came into a room and started talking to her/him without calling her/his name, did she/he usually look up and pay attention to you?	**.65**		.42
39. When she/he was 4 to 5, did she/he ever play imaginative games with another child in such a way that you could tell that each child understood what the other was pretending?	**.67**		.45
40. When she/he was 4 to 5, did she/he play cooperatively in games that required joining in with a group of other children, such as hide-and-seek or ball games?	**.70**		.50

Note: Factor loadings below .25 are suppressed

A single factor solution found that 12 questions loaded moderately (i.e. factor loadings above .50) onto this factor. These factor loadings are shown in Table 10. As both models generally showed only moderate loading scores, but the single factor model logically included questions which all measured social communication and interaction, while the two factor model arbitrarily split related questions across factors, the single factor model was selected.

The items that loaded moderately in this model were averaged to provide a "Social Communication and Interaction (SCQ-Lifetime)" score for each participant.

Table 10.
Factor loadings and communalities based on principal component analysis with varimax rotation for the SCQ-Lifetime for a forced one factor solution after removing all items not relating to social communication and interaction

	Factor 1: Social Communication and Interaction (SCQ)	Communality
9. Has her/his facial expression usually seemed appropriate to the particular situation, as far as you could tell?	.35	.12

10. Has she/he ever used your hand like a tool or as if it were a part of her/his own body (e.g. pointing with your finger or putting your hand on a doorknob to get you to open the door)?	.43	.19
20. When she/he was 4 to 5, did she/he ever talk with you just to be friendly (rather than to get something)?	**.61**	.37
22. When she/he was 4 to 5, did she/he ever spontaneously point at things around her/him just to show you things (not because she/he wanted them)?	.46	.21
23. When she/he was 4 to 5, did she/he ever use gestures, other than pointing or pulling your hand, to let you know what she/he wanted?	.39	.15
24. When she/he was 4 to 5, did she/he nod her/his head to mean *yes*?	.37	.14
25. When she/he was 4 to 5, did she/he shake her/his head to mean *no*?	.41	.17
26. When she/he was 4 to 5, did she/he usually look at you directly in the face when doing things with you or talking with you?	**.52**	.27
27. When she/he was 4 to 5, did she/he smile back if someone smiled at her/him?	**.63**	.39
28. When she/he was 4 to 5, did she/he ever show you things that interested him/her to engage your attention?	.46	.21
29. When she/he was 4 to 5, did she/he ever offer to share things other than food with you?	**.56**	.32
30. When she/he was 4 to 5, did she/he ever seem to want you to join in her/his enjoyment of something?	**.59**	.34
31. When she/he was 4 to 5, did she/he ever try to comfort you if you were sad or hurt?	**.57**	.33
33. When she/he was 4 to 5, did she/he show a normal range of facial expressions?	**.65**	.42
36. When she/he was 4 to 5, did she/he seem interested in other children of approximately the same age whom she/he did not know?	**.60**	.36
37. When she/he was 4 to 5, did she/he respond positively when another child approached her/him?	**.67**	.44

38. When she/he was 4 to 5, if you came into a room and started talking to her/him without calling her/his name, did she/he usually look up and pay attention to you?	**.54**	.30
39. When she/he was 4 to 5, did she/he ever play imaginative games with another child in such a way that you could tell that each child understood what the other was pretending?	**.51**	.26
40. When she/he was 4 to 5, did she/he play cooperatively in games that required joining in with a group of other children, such as hide-and-seek or ball games?	**.55**	.30

Note: Factor loadings below .25 are suppressed

Repetitive Behavior Scale – Revised (Lam & Aman, 2007). The Repetitive Behavior Scale – Revised mainly focused on restricted and repetitive behaviours and interests, where higher scores indicated a greater deficit (Lam & Aman, 2007). 67 children had complete data for the RBS-R. This protocol utilised the alternative 5-subscale scoring solution for the RBS-R as detailed by Lam (2004). Internal consistency was assessed with Cronbach's alpha and found the following scores: Total Score (Cronbach's α =.77), Restricted Interests (Cronbach's α =.68), Ritualistic / Sameness Behaviour (Cronbach's α =.65), Stereotyped Behaviours (Cronbach's α =.63), Compulsive Behaviours (Cronbach's α =.60), and Self-Injurious Behaviours (Cronbach's α =.54). These subscales do not reflect the symptom dimensions as described in either the DSM-5 or DSM-IV-TR, which both describe restricted and repetitive behaviours as a single symptom dimension without subtypes. The measure was assessed for questions with weak correlation to the overall Cronbach's alpha (that is, less than $r = .30$) and these were removed. Of the 43 items, 4 were identified as weak and were eliminated (Appendix M).

Principal component factor analysis with varimax rotation was run on the remaining 39 questions. The Kaiser-Meyer-Olkin measure was slightly low but indicated that the sample size was adequate for factor analysis on this data, KMO = .69 (Field, 2009). As this value was low and the sample was smaller than the generally accepted standard of 10-15 participants per variable, these results must be interpreted with caution. Bartlett's Test of Sphericity indicated sufficiently large correlations between questions for principal component factor analysis, $\chi^2 (741) = 1809.85$, $p < .001$. Initial analysis indicated 10 components with eigenvalues over

Kaiser's criterion of 1, and together explained 72.89% of the total variance. The scree plot indicated a point of inflection that justified retaining 2 components (19.79% total variance).

The RBS-R has five given subscales. As the scree plot did not support a five factor model, Cronbach's alpha found weaknesses with the given subscales, and the subscales do not reflect the diagnostic criteria of either the DSM-IV-TR or DSM-5, they were not considered reliable for this sample.

All questions on the RBS-R measure restricted and repetitive behaviours and interests, a single symptom dimension on both the DSM-IV-TR and DSM-5, so a single factor model was also assessed. This model was also assessed in case restricted interests and repetitive behaviours loaded separately despite being a single symptom domain in the DSM-5. The scree plot is shown in Figure 7.

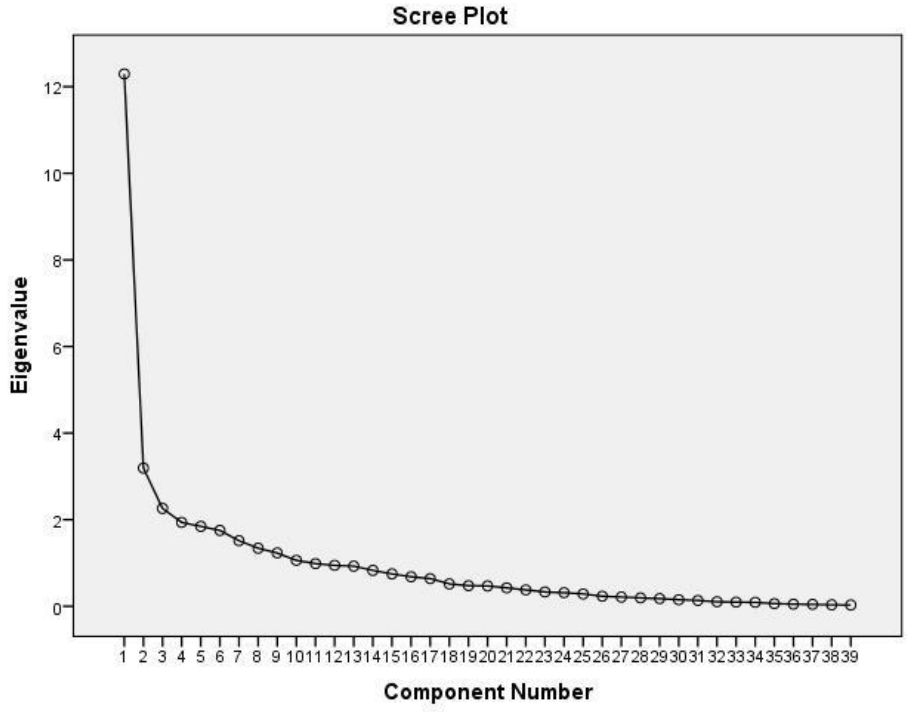

Figure 7. Scree plot based on principal component analysis with varimax rotation for the RBS-R

Principal component factor analysis with varimax rotation was run forcing a 2 factor solution. This solution explained 39.71% of the total variance. The factor loadings for the two factor model are shown in Table 11. An assessment of these loadings did not reveal a logical underlying structure. Many items did not load onto either factor. Most notable, many items

from the Compulsive Behaviour and the Restricted Behaviour subscales did not load onto either factor, leading to underrepresentation of these aspects of this core symptom domain.

Table 11.

Factor loadings and communalities based on principal component analysis with varimax rotation for the RBS-R for a forced two factor solution

	Factor 1	Factor 2	Communality
1. WHOLE BODY (body rocking, body swaying)		**.65**	.42
2. HEAD (rolls head, nods head, turns head)		**.65**	.44
4. LOCOMOTION (turns in circles, whirls, jumps, bounces)		**.64**	.42
5. OBJECT USAGE (spins or twirls objects, twiddles or slaps or throws objects, lets objects fall out of hands)		**.72**	.56
6. SENSORY (covers eyes, looks closely or gazes at hands or objects, covers ears, smells or sniffs items, rubs surfaces)		**.54**	.30
7. HITS SELF WITH BODY PART (hits or slaps head, face, or other body area)		**.56**	.34
8. HITS SELF AGAINST SURFACE OR OBJECT (hits or bangs head or other body part on table, floor or other surface)		.46	.25
9. HITS SELF WITH OBJECT (hits or bangs head or other body area with objects)		**.53**	.30
12. RUBS OR SCRATCHES SELF (rubs or scratches marks on arms, leg, face or torso)		**.62**	.38
14. SKIN PICKING (picks at skin on face, hands, arms, legs or torso)			.09
15. ARRANGING/ORDERING (arranges certain objects in a particular pattern or place; need for things to be even or symmetrical)	**.52**	.37	.40
16. COMPLETENESS (must have doors opened or closed; takes all items out of a container or area)	**.54**	.35	.41
17. WASHING/CLEANING (excessively cleans certain body parts; picks at link or loose threads)	.42		.20

18. CHECKING (repeatedly checks doors, windows, drawers, appliances, clocks, locks, etc)		.42	.21
19. COUNTING (counts items or objects; counts to a certain number or in a certain way)		.46	.24
20. HOARDING/SAVING (collects, hoards or hides specific items)	.49	.28	.31
21. REPEATING (need to repeat routine events; in/out door, up/down from chair, clothing on/off)	**.54**	.37	.42
22. TOUCH/TAP (need to touch, tap, or rub items, surfaces, or people)	.38	.49	.39
23. EATING/MEALTIME (strongly prefers/insists on eating/drinking only certain things; eats or drinks items in a set order; insists that meal related items are arranged in a certain way)	**.55**		.31
24. SLEEPING/BEDTIME (insists on certain pre-bedtime routines; arranges items in room "just so" prior to bedtime; insists that certain items be present with him/her during sleep; insists that another person be present prior to or during sleep)	**.61**	.34	.48
25. SELF-CARE – BATHROOM AND DRESSING (insists on specific order of activities or tasks related to using the bathroom, to washing, showering, bathing or dressing; arranges items in a certain way in the bathroom or insists that bathroom items not be moved; insists on wearing certain clothing items)	**.71**		.52
26. TRAVEL/TRANSPORTATION (insists on taking certain routes/paths; must sit in specific location in vehicles; insists that certain items be present during travel, e.g. toy or material; insists on seeing or touching certain things or places during travel, such as a sign or store)	**.55**		.31
27. PLAY/LEISURE (insists on certain play activities; follows a rigid routine during play/leisure; insists that certain items be present/available during play/leisure; insists that other persons do certain things during play)	**.61**	.27	.44
28. COMMUNICATION/SOCIAL INTERACTIONS (repeats same	**.56**		.36

topic(s) during social interactions; repetitive questioning; insists on certain topics of conversation; insists that others say certain things or respond in certain ways during interactions)			
29. Insists that things remain in the same place(s) (e.g. toys, supplies, furniture, pictures, etc)	**.76**		.63
30. Objects to / resists visiting new places	**.53**		.30
31. Becomes upset if interrupted in what he/she is doing	**.56**	.28	.38
32. Insists on walking in a particular pattern (e.g. straight line)	**.63**		.43
33. Insists on sitting at the same place	**.79**		.62
34. Disliked changes in appearance or behaviour of the people around him/her	**.69**		.50
35. Insists on using a particular door	.49	.32	.34
36. Likes the same CD, take, record or piece of music played continually; likes same movie/video or part of movie/video	**.69**		.54
37. Resists changing activities; difficulty with transitions	**.67**	.32	.55
38. Insists on same routine, household, school or work schedule everyday	**.72**	.28	.60
39. Insists that specific things take place as specific times	**.59**		.38
40. Fascination, preoccupation with one subject or activity (e.g. trains, computers, weather, dinosaurs)	.33	.42	.29
41. Strongly attached to one specific object	.48	.47	.45
42. Preoccupation with part(s) of objects rather than the whole object (e.g. buttons on clothes, wheels on toy cars)	.33	**.61**	.49
43. Fascination, preoccupation with movement / things that move (e.g. fans, clocks)		**.64**	.45

Note: Factor loadings below .25 are suppressed

Principal component factor analysis forcing a one factor solution found that a single factor explained 31.52% of the total variance. The factor loadings for this model are shown in Table 12. Some items loaded poorly, such as “CHECKING (repeatedly checks doors,

windows, drawers, appliances, clocks, locks, etc)".and "SKIN PICKING (picks at skin on face, hands, arms, legs or torso)". However, the majority of the questions (27 of the retained 39 questions) loaded moderately to strongly (i.e. factor loadings above .50) onto this factor.

Table 12.

Factor loadings and communalities based on principal component analysis with varimax rotation for the RBS-R for a forced two factor solution

	Factor 1: Restricted and Repetitive Behaviours and Interests (RBS-R)	Communality
1. WHOLE BODY (body rocking, body swaying)	.38	.14
2. HEAD (rolls head, nods head, turns head)	**.50**	.25
4. LOCOMOTION (turns in circles, whirls, jumps, bounces)	.47	.22
5. OBJECT USAGE (spins or twirls objects, twiddles or slaps or throws objects, lets objects fall out of hands)	**.60**	.36
6. SENSORY (covers eyes, looks closely or gazes at hands or objects, covers ears, smells or sniffs items, rubs surfaces)	.40	.16
7. HITS SELF WITH BODY PART (hits or slaps head, face, or other body area)	.46	.21
8. HITS SELF AGAINST SURFACE OR OBJECT (hits or bangs head or other body part on table, floor or other surface)	.45	.20
9. HITS SELF WITH OBJECT (hits or bangs head or other body area with objects)	.43	.19
12. RUBS OR SCRATCHES SELF (rubs or scratches marks on arms, leg, face or torso)	.38	.15
14. SKIN PICKING (picks at skin on face, hands, arms, legs or torso)	.30	.09
15. ARRANGING/ORDERING (arranges certain objects in a particular pattern or place; need for things to be even or symmetrical)	**.63**	.40
16. COMPLETENESS (must have doors opened or closed; takes all items out of a container or area)	**.64**	.41
17. WASHING/CLEANING (excessively cleans certain body parts; picks at link or loose threads)	.42	.18

18. CHECKING (repeatedly checks doors, windows, drawers, appliances, clocks, locks, etc)	.39	.15
19. COUNTING (counts items or objects; counts to a certain number or in a certain way)	.41	.17
20. HOARDING/SAVING (collects, hoards or hides specific items)	**.56**	.31
21. REPEATING (need to repeat routine events; in/out door, up/down from chair, clothing on/off)	**.65**	.42
22. TOUCH/TAP (need to touch, tap, or rub items, surfaces, or people)	**.60**	.36
23. EATING/MEALTIME (strongly prefers/insists on eating/drinking only certain things; eats or drinks items in a set order; insists that meal related items are arranged in a certain way)	.39	.15
24. SLEEPING/BEDTIME (insists on certain pre-bedtime routines; arranges items in room "just so" prior to bedtime; insists that certain items be present with him/her during sleep; insists that another person be present prior to or during sleep)	**.69**	.48
25. SELF-CARE – BATHROOM AND DRESSING (insists on specific order of activities or tasks related to using the bathroom, to washing, showering, bathing or dressing; arranges items in a certain way in the bathroom or insists that bathroom items not be moved; insists on wearing certain clothing items)	**.51**	.26
26. TRAVEL/TRANSPORTATION (insists on taking certain routes/paths; must sit in specific location in vehicles; insists that certain items be present during travel, e.g. toy or material; insists on seeing or touching certain things or places during travel, such as a sign or store)	**.50**	.25
27. PLAY/LEISURE (insists on certain play activities; follows a rigid routine during play/leisure; insists that certain items be present/available during play/leisure; insists that other persons do certain things during play)	**.65**	.42

28. COMMUNICATION/SOCIAL INTERACTIONS (repeats same topic(s) during social interactions; repetitive questioning; insists on certain topics of conversation; insists that others say certain things or respond in certain ways during interactions)	**.58**	.33
29. Insists that things remain in the same place(s) (e.g. toys, supplies, furniture, pictures, etc)	**.74**	.55
30. Objects to / resists visiting new places	**.51**	.26
31. Becomes upset if interrupted in what he/she is doing	**.61**	.37
32. Insists on walking in a particular pattern (e.g. straight line)	**.62**	.39
33. Insists on sitting at the same place	**.63**	.40
34. Disliked changes in appearance or behaviour of the people around him/her	**.65**	.42
35. Insists on using a particular door	**.59**	.34
36. Likes the same CD, take, record or piece of music played continually; likes same movie/video or part of movie/video	**.70**	.49
37. Resists changing activities; difficulty with transitions	**.73**	.53
38. Insists on same routine, household, school or work schedule everyday	**.74**	.55
39. Insists that specific things take place as specific times	**.58**	.33
40. Fascination, preoccupation with one subject or activity (e.g. trains, computers, weather, dinosaurs)	**.52**	.27
41. Strongly attached to one specific object	**.66**	.44
42. Preoccupation with part(s) of objects rather than the whole object (e.g. buttons on clothes, wheels on toy cars)	**.63**	.40
43. Fascination, preoccupation with movement / things that move (e.g. fans, clocks)	**.53**	.29

Note: Factor loadings below .25 are suppressed

The two factor model was rejected in favour of the single factor model. Scores for all final 27 retained questions were averaged to give each participant a score for "Restricted and Repetitive Behaviours and Interests (RBS-R)".

Correlations between ASD characteristic scores. Correlation analysis was conducted between the new scores for participants (i.e. average scores on the retained item sets for each scale). These formed the composite scores Social Communication and Interaction (ASSP), Social Communication and Interaction (SCQ-Lifetime), and Restricted and Repetitive Behaviours and Interests (RBS-R).

To qualify for a DSM-5 ASD diagnosis, children must present with deficits in both domains, but these areas of deficit continue to be conceptualized as relatively independent of each other. It was therefore expected that Social Communication and Interaction (ASSP) and Social Communication and Interaction (SCQ-Lifetime) should correlate strongly as they assess the same symptom domain, while neither would correlate as strongly to Restricted and Repetitive Behaviours and Interests (RBS-R).

Spearman's rho found significant correlations between Social Communication and Interaction (ASSP) and Social Communication and Interaction (SCQ-Lifetime), $r_s = .49$, $p < .001$, and between Social Communication and Interaction (ASSP) and Restricted and Repetitive Behaviours and Interests (RBS-R), $r_s = .52$, $p < .001$. The correlation between Social Communication and Interaction (SCQ-Lifetime) and Restricted and Repetitive Behaviours and Interests (RBS-R) was approaching significance, $r_s = .23$, $p = .06$.

Although Social Communication and Interaction (ASSP) and Social Communication and Interaction (SCQ-Lifetime) were both used as measures of social communication and interaction, the scores were not merged as they had different scales of measure (likert scale versus a yes-no format), and because the scores did not correlate highly.

These core ASD symptom scores were then used to in Study One to assess whether 5-HTTLPR was related to symptom presentation in ASD, and in Study Two to assess for possible relationships between core ASD symptoms, 5-HTTLPR and ToM.

Study One: ASD Phenotypes for 5-HTTLPR Genotypes

Study One compared Social Communication and Interaction (ASSP), Social Communication and Interaction (SCQ-Lifetime) and Restricted and Repetitive Behaviours and Interests (RBS-R) across 5-HTTLPR genotypes. 55 participants were successfully genotyped and had provided sufficient information regarding their core ASD symptoms. Their demographic details were discussed above. Not all participants returned all three symptom

scales, but these participants were still included in the analyses which used data from the scales they did return (i.e. if a child returned the ASSP and SCQ, but not the RBS-R, they were only excluded from analyses that included RBS-R data). Study One aimed to assess whether 5-HTTLPR genotypes were related to core symptoms in ASD.

Core ASD symptoms across 5-HTTLPR genotypes. Core ASD symptoms were represented by composite scores calculated from retained item sets from the ASSP, RBS-R, and SCQ-Lifetime. This resulted in each participant having the following scores: Social Communication and Interaction (ASSP), Social Communication and Interaction (SCQ-Lifetime), and Restricted and Repetitive Behaviours and Interests (RBS-R). Higher scores indicated greater impairment.

These scores were compared across genotype. The short allele has reduced transcription compared to the long allele. The transcriptional efficacy of the genotypes is therefore best for the *l/l* genotype, and most reduced in the *s/s* genotype. However, some debate exists around the dominance of the short allele, suggesting that the *l/s* genotype and *s/s* genotype may have similar rates of transcription (Tordjman et al., 2001). Scores were therefore also compared across alleles, with the *l/l* genotype forming a "normal transcription" group and the *l/s* genotype and *s/s* genotype collectively forming a "reduced transcription" group.

Based on previous findings in the literature, we hypothesised that children carrying the long allele would present with higher impairment in restricted and repetitive behaviours and interests, while those with the short allele would present with greater impairment in social communication and interaction.

Social Communication and Interaction (ASSP), Social Communication and Interaction (SCQ-Lifetime), and Restricted and Repetitive Behaviours and Interests (RBS-R) scores were compared across genotypes with a MANOVA. MANOVA is robust against unequal group sizes when all other assumptions are met (Field, 2009). For this analyses observations were independent, data was measured at the interval level or above, and homogeneity of covariance matrices was upheld (as indicated by Box's M = 6.26, F (12, 9307.71) = .47, p = .93). Using Pillai's trace, there was not a significant effect for genotype on ASD symptoms, V= 0.05, F (6, 98) = 0.44, p = .85, η^2 = .03.

ANOVAs were then run to individually assess each symptom score across genotypes, and no significant between-group differences were found. ANOVA is robust against unequal group sizes when all other assumptions are met (Field, 2009). For the following analyses these assumptions were met: observations were independent, the dependent variable was at

least of the interval scale, and the group variances were not significantly different. Mean scores and ANOVA results are shown in Table 13.

Table 13.
ANOVA Results for core ASD Symptoms across 5-HTTLPR genotypes

	l/l Genotype	*l/s* Genotype	*s/s* Genotype	Homogeneity of variance (*p*)	F	*p*	*r*
N	18	21	14				
Social Communication and Interaction (ASSP)				.66	0.26	.77	.10
Mean	2.72	2.86	2.78				
SD	0.52	0.59	0.52				
Social Communication and Interaction (SCQ-Lifetime)				.28	0.18	.83	.08
Mean	0.62	0.57	0.57				
SD	0.29	0.24	0.32				
Restricted and Repetitive Behaviours and Interests (RBS-R)				.66	0.09	.92	.06
Mean	0.84	0.76	0.76				
SD	0.61	0.64	0.49				

It was interesting to note that the *l/s* and *s/s* genotypes scored identical mean values for the Social Communication and Interaction (SCQ-Lifetime) and Restricted and Repetitive Behaviours and Interests (RBS-R). As both these genotypes contain short alleles, this suggested that the role of 5-HTTLPR on ASD symptoms may be due to alleles rather than genotype – that is, those with a short allele present differently to those with only long alleles.

Core ASD symptoms across 5-HTTLPR alleles. In the above analyses, core ASD symptoms were assessed across the three genotypes with the assumption that the genotypes have varying degrees of serotonergic transmission (that is, that the *l/l* genotype has the best efficacy, the *l/s* reduced efficacy, and the *s/s* the most reduced efficacy). Following the trend noted in the literature (Brune et al., 2006; Tordjman et al., 2001), the following analyses compared core ASD symptoms across alleles such that the genotypes containing short alleles

(namely, *l/s* and *s/s*) were grouped in a single "reduced transcription" group and were compared to the *l/l* genotype which was seen as a "normal transcription" group. The normal transcription group consisted of 18 participants and the reduced transcription group consisted of 36 participants (except for Restricted and Repetitive Behaviours and Interests (RBS-R), which had 35 participants). The hypotheses remained the same as in the above analyses: we hypothesised that the normal transcription group (i.e. only carry long alleles) would present with greater impairment in restricted and repetitive behaviours and interests, while the children in the reduced transcription group (i.e. carry at least one short allele) would present with greater impairment in social communication and interaction.

Social Communication and Interaction (ASSP), Social Communication and Interaction (SCQ-Lifetime), and Restricted and Repetitive Behaviours and Interests (RBS-R) scores were compared across alleles using an ANOVA (see Table X for results). ANOVA is robust against unequal group sizes when all other assumptions are met (Field, 2009). For these analyses these assumptions were met: observations were independent, the dependent variable was at least of the interval scale, and the group variances were not significantly different. ANOVA found no significant between-group differences. Mean scores and ANOVA results are shown in Table 14.

The reduced transcription group was expected to score higher compared to the normal transcription group, indicating greater deficit, but this result was not found for any of the symptoms measured. It is noted that scores for Social Communication and Interaction (ASSP) were almost identical between groups. However, considering the ASSP measures characteristics on a four point likert scale, the standard deviations were high. A larger sample size is necessary to explore whether the similarities in social skills and communication as assessed by the ASSP are a true reflection of ability.

Between-group differences on Social Communication and Interaction (SCQ-Lifetime) and Restricted and Repetitive Behaviours and Interests (RBS-R) were minimal, not significant and effect sizes were small. The small sample size is limiting, and the small effect sizes suggest that larger sample sizes might not change the findings.

Overall, mean scores hardly varied across groups, suggesting that 5-HTTLPR alleles do not influence the presentation of core ASD symptoms, as assessed in the current protocol.

Table 14.
ASD Symptoms Across Alleles

	Normal Transcription	Reduced Transcription	Homogeneity of variance (*p*)	F	*p*	*r*
Social Communication and Interaction (ASSP)			.43	< 0.01	.96	.01
N	18	36				
Mean	2.79	2.78				
SD	0.46	0.59				
Social Communication and Interaction (SCQ-Lifetime)			.91	0.37	.54	.08
N	18	36				
Mean	0.62	0.57				
SD	0.29	0.27				
Restricted and Repetitive Behaviours and Interests (RBS-R)			.75	0.18	.67	.06
N	18	35				
Mean	0.84	0.76				
SD	0.61	0.58				

Core ASD symptoms across verbal and non-verbal participants. The study was purposefully designed to include non-verbal participants. Twelve non-verbal children and 57 verbal children participated in the study, although only 56 completed the symptom scales, and one of those failed to return data for the RBS-R. Children were included in the non-verbal group if they were unable to follow simple commands and/or verbalise responses. Some of these children did have limited language use and did not have pure non-verbal clinical presentations. Scores for Social Communication and Interaction (ASSP), Social Communication and Interaction (SCQ-Lifetime) and Restricted and Repetitive Behaviours and Interests (RBS-R) were compared between verbal and non-verbal participants. Due to the very small size of the non-verbal group, these scores are discussed qualitatively. The mean scores for each group are shown in Table 15.

Table 15.
Core ASD Symptoms Across Non-verbal and Verbal Participants

	Verbal	Non-Verbal
Social Communication and Interaction (ASSP)		
N	56	12
Mean	2.73	3.20
SD	0.51	0.34
Social Communication and Interaction (SCQ-Lifetime)		
N	56	12
Mean	0.58	0.65
SD	0.30	0.24
Restricted and Repetitive Behaviours and Interests (RBS-R)		
N	55	12
Mean	0.77	1.06
SD	0.58	0.62

The non-verbal group had higher scores across all three measures. As higher scores indicate greater impairment, this was expected. The RBS-R used a four point likert scale, and the SCQ-Lifetime used a Yes/No format. For this protocol, the ASSP rated characteristics between one and four, the SCQ-Lifetime between zero and one, and the RBS-R between zero and three. Keeping the possible range of scores in mind, we noted that the disparity in Social Communication and Interaction (ASSP) is greatest, but Social Communication and Interaction (SCQ-Lifetime) has the smallest between-group difference. ASD symptoms exist on a dimension of severity, and these findings suggest that the likert scale used in the ASSP to assess the severity of a range of social skills and communication may be more meaningful than the SCQ-Lifetime's measure of whether the skills are absent or present. This difference may make the ASSP more sensitive to subtle differences in social skills and communication within an ASD sample than the SCQ-Lifetime.

Core ASD symptoms across genotypes for non-verbal participants. Nine of the 12 non-verbal children who participated in the study were successfully genotyped. Of these 9 participants, 2 presented with the *l/l* genotype, 7 with the *l*/s genotype, and none presented with the *s/s* genotype. It was unsurprising that most of the group carried a short allele as we expected this allele, with its reduced transcriptional efficacy, to be more prevalent in the ASD population, although we had expected a higher incidence of the *s/s* genotypes. Social Communication and Interaction (ASSP), Social Communication and Interaction (SCQ-

Lifetime) and Restricted and Repetitive Behaviours and Interests (RBS-R) scores within this group are shown in Table 16. Due to the small sample size, these are discussed qualitatively.

Table 16.
Core ASD Symptoms Across Genotypes for Non-verbal Participants

	l/l	*l/s*
N	2	7
Social Communication and Interaction (ASSP)		
Mean	2.94	3.23
SD	0.52	0.38
Social Communication and Interaction (SCQ-Lifetime)		
Mean	0.75	0.52
SD	0.24	0.21
Restricted and Repetitive Behaviours and Interests (RBS-R)		
Mean	1.14	0.92
SD	0.67	0.76

Social Communication and Interaction (ASSP) showed the expected pattern that the children with reduced transcription (i.e. the *l/s* genotype) had higher scores (i.e. greater impairment). However, Social Communication and Interaction (SCQ-Lifetime) showed the reverse pattern, with those with normal serotonergic transmission showing greater impairment. This contradiction between Skills and Communication (ASSP) and Social Communication and Interaction (SCQ-Lifetime) scores across genotypes could be due to the different type of measurement used in the ASSP and SCQ-Lifetime. As the SCQ-Lifetime assesses presence of deficit on a Yes/No scale while the ASSP uses a likert scale, this could indicate that children with the *l/l* genotype could have a higher incidence of impairment in social communication and interaction, while those with the *l/s* genotype have greater severity when the impairment is present.

The difference in scores for the normal transcription group and the reduced transcription group for Restricted and Repetitive Behaviours and Interests (RBS-S) is minimal, with both groups having standard deviations indicating that scores are quite varied in each group. This suggests that genotypes do not influence scores for Restricted and Repetitive Behaviours and Interests (RBS-R).

Study One results summary. Study One explored possible relationships between 5-HTTLPR genotypes and core ASD symptoms with MANOVA and found no significant

between-group differences. Assessment of group means showed that the *l/s* and *s/s* group presented with the same mean scores for Social Communication and Interaction (SCQ-Lifetime) and Restricted and Repetitive Behaviours and Interests (RBS), which suggested allele, rather than genotype, influenced ASD presentations.

Study One then explored possible differences in core ASD symptoms across alleles, such that the *l/l* genotype formed a "normal transcription" group and the *l/s* and *s/s* genotypes were collectively grouped as the "reduced transcription" group. ANOVA found no significant between-group differences. Social Communication and Interaction (ASSP) scored almost equally across groups, while group differences in mean scores for Social Communication and Interaction (SCQ-Lifetime) and Restricted and Repetitive Behaviours and Interests (RBS-R) were minimal, suggesting the 5-HTTLPR alleles do not influence the core symptom domains in ASD as measured in this protocol.

Study One also explored descriptive differences in core ASD symptoms between verbal and non-verbal participants. The non-verbal group showed somewhat greater deficits across all three measures. This disparity was greatest for Social Communication and Interaction (ASSP), but smallest for Social Communication and Interaction (SCQ-Lifetime). The ASSP uses a likert scale to assess for the severity of impairment, while the SCQ-Lifetime focuses on whether certain skills are present or absent. The ASSP may be more sensitive to subtle differences in social skills and communication within an ASD sample than the SCQ-Lifetime. Although the findings were limited by the small sample size, they suggest that non-verbal children with ASD present with greater deficits in both core symptom domains of the DSM-5, namely Social Skills and Communication and Restricted and Repetitive Behaviours and Interests (American Psychiatric Association, 2013).

Study One's final investigation focused on core ASD symptoms for non-verbal participants across genotypes. Within this very small sample it was descriptively observed that children with reduced serotonergic transmission had increased impairment in Social Communication and Interaction (ASSP), but not in the other two domains. All scores were high, however, again suggesting that non-verbal children with ASD have high levels of impairment in both core symptom domains independently of 5-HTTLPR genotype.

Core ASD symptoms, as assessed by the three scales used in this protocol, appear to be independent of serotonergic transmission as mediated by 5-HTTLPR.This was found for verbal and non-verbal participants. Our hypotheses that the long allele was would be associated with increased impairment in restricted and repetitive behaviours and interests and

the short allele would be associated with increased impairment in social communication and interaction were not supported.

Study Two: Core ASD Symptoms, 5-HTTLPR Genotypes, and Theory of Mind

ToM scores. 57 children underwent ToM testing, and of these 46 were successfully genotyped, resulting in a sample of 57 for the exploration of possible relationships between core ASD symptoms and ToM, and a sample of 46 for the exploration of possible relationships between ToM and 5-HTTLPR.

Kolmogorov-Smirnov analysis revealed that the ToM total scores were not normally distributed, D (55) = 0.12, $p < .05$, M = 26.07, SD = 16.87.

Core ASD symptoms and ToM. The possible relationships between ToM ability and core ASD symptoms were assessed using one-tailed correlation analysis at alpha = .05. We expected to find correlations between ToM deficits and deficits in areas related to social ability. As higher ToM scores indicated higher ToM ability, while higher scores on symptom measures indicate greater impairment in that area, we expected negative correlation scores.

As ToM scores were not normally distributed, Spearman's rho was used to assess correlations between Total ToM score and Social Communication and Interaction (ASSP), Social Communication and Interaction (SCQ-Lifetime) and Restricted and Repetitive Behaviours and Interests (RBS-R). ToM did not correlate significantly with any of the core ASD symptoms, as is shown in Table 17. The relationships between ToM ability and core ASD symptoms assessed in this protocol remain unclear, and are limited by the measures used to assess ASD symptoms.

Table 17.

Correlations Between ToM Scores and ASD Symptom Scores

	ToM
Social Communication and Interaction (ASSP)	$r_s < .01$, $p = .49$
Social Communication and Interaction (SCQ-Lifetime)	$r_s = -.12$, $p = .18$
Restricted and Repetitive Behaviours and Interests (RBS-R)	$r_s = .18$, $p = .09$

ToM and age. The role of age in ToM ability was explored. In typically developing children age correlates positively with ToM ability, so one tailed correlation was assessed using Spearman's rho. ToM and age showed a positive correlation, $r_s = .48$, $p < .001$.

ToM ability, as indicated by ToM total scores, was assessed across age for all participants in Study Two, and is shown in Figure 8. Although age accounts for 18.90% of the variability in ToM, no clear developmental pattern emerged.

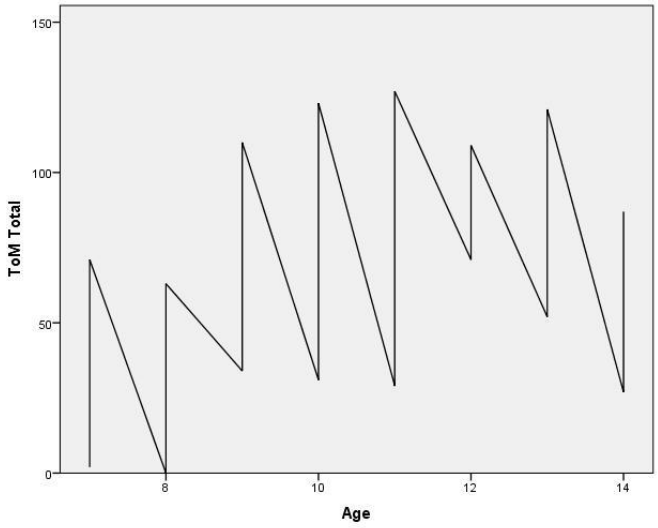

Figure 8. ToM ability across age for all participants from Study Two

ToM and age across 5-HTTLPR genotypes. Total ToM scores were assessed across 5-HTTLPR genotypes. The 46 participants for this study presented the following genotype distribution: 16 *l/l* genotypes, 16 *l/s* genotypes, and 14 *s/s* genotypes. We hypothesised that the children who carried short alleles would have poorer ToM ability than those with only long alleles as the literature showed an association between the short allele and greater impairment in social communication and interaction (Brune et al., 2006; Tordjman et al., 2001), and ToM ability correlates with social competence in typically developing children (Bosacki & Wilde Astington, 2001; Repacholi & Slaughter, 2003).

The possibility that 5-HTTLPR genotypes would meaningfully identify groups of children with different ToM abilities was considered – that is, that different genotypes would present different ToM abilities. ToM was plotted against age for each genotype and is shown in Figure 10. No differences in start of ToM development or rate of ToM development were found, and no genotypes showed a plateau in ToM development.

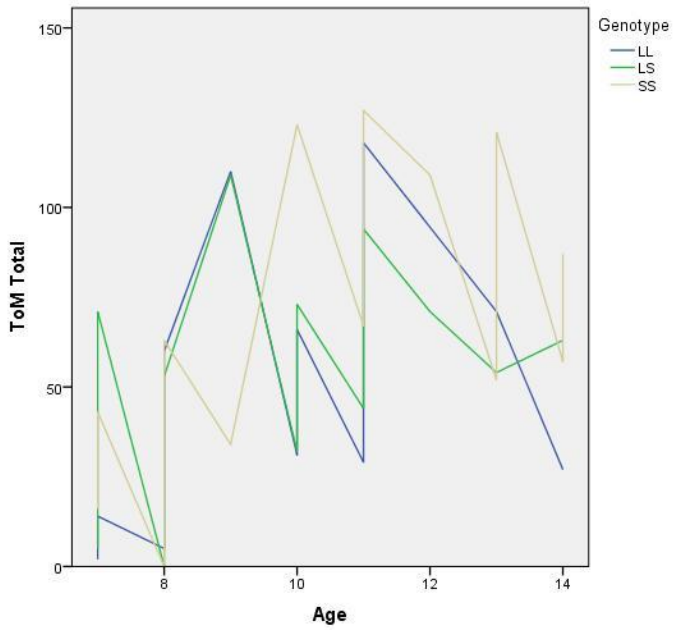

Figure 10. ToM ability across age for each 5-HTTLPR genotype.

It was theorised that genotypes would predispose individuals to different patterns of ToM development, and that age would then influence how far a child was in this development. A hierarchical multiple regression was run using age and genotype as the predictors and ToM as the outcome variable. Genotype was entered first, and then age was accounted for. As there were three possible genotypes, dummy variables were used. The assumptions that both dependent variables were continuous and independent of each other were met, and multicolinearity and homoscedascity were assessed and were not problematic (Field, 2009).

The resulting regression model was statistically significant and explained 19.50% of the variance in ToM scores. *Genotype* and *Age* significantly predict *ToM*, $R = .47$, $R^2 = .22$, $F(3, 42) = 3.91$, $p = .015$. The model parameters are shown in Table 18. The Model Summary is shown in Table 19.

From the Model Summary we note that *Genotype* and did not significantly predict *ToM*, $R = .76$, $R^2 = .03$, $F(2, 43) = 0.68$, $p = .52$. When *Age* was added to the model, a further 19.00% of the variance in *ToM* was explained, and this model was significant, $R = .47$, $R^2 = .22$, $F(1, 42) = 10.08$, $p < .01$. This result indicated that age mainly accounted for the effect, and genotype did not affect ToM ability.

Table 18.

Multiple Regression for Age, Genotype, and ToM

	B	*b*	SE	t (42)	*p*
Constant	-	-15.73	22.79	-0.69	.49
Genotype 1	.04	3.34	11.75	0.29	.78
Genotype 2	.13	10.25	12.19	0.84	.41
Age	.44	6.92	2.18	3.18	.003

Note: As genotype had three categories, dummy variables were used such that the l/l genotype formed the control condition

Table 19.

Model Summary for Hierarchical Multiple Regression for Age, Genotype, and ToM

Model	*R*	*R Square*	Adj. *R Square*	Std. Error of Est.	*R Square Change*	*F Change*	Df 1	Df 2	Sig. *F Change*
Genotype	.76	.03	-.02	36.48	.03	0.68	2	43	.52
Genotype, Age	.47	.22	.16	33.14	.19	10.08	1	42	<.01

ToM and age across 5-HTTLPR alleles. Participants were then grouped by allele into Normal Transcription and Reduced Transcription groups to assess whether allele, rather than genotype, influenced ToM across age.

A hierarchical multiple regression was run using age and allele as the predictors and ToM as the outcome variable. Allele was entered first, followed by age. The resulting regression model was statistically significant and explained 21.80% of the variance in ToM scores. *Age* and *Allele* significantly predict *ToM*, $R = .46$, $R^2 = .21$, $F(2, 43) = 5.79$, $p = .006$. The regression equation derived from this model is *ToM* = -24.22 + 6.56 * *Allele* + 7.11 * *Age*. The model parameters are shown in Table 20. The Model Summary is shown in Table 22.

From the Model Summary we note that *Allele* and did not significant predict *ToM*, $R = .09$, $R^2 = .01$, $F(1, 44) = 0.38$, $p = .54$. When *Age* was added to the model, a further 20.00% of the variance in *ToM* was explained, and this model was significant, $R = .46$, $R^2 = .21$, $F(1, 43)$

= 11.13, $p < .01$. This result indicated that age mainly accounted for the effect, and genotype did not affect ToM ability.

The small variability accounted for by genotype and / or allele, as well as the absence of developmental differences in ToM ability across genotypes, suggests that 5-HTTLPR did not influence ToM ability or development in this sample.

Table 20.

Multiple Regression for Age, Allele, and ToM

	β	*b*	SE	t (43)	*p*
Constant	-	-24.22	27.09	-0.89	.38
Allele	.09	6.56	10.18	0.64	.52
Age	.45	7.11	2.13	3.34	.002

Table 21.

Model	*R*	*R Square*	Adj. *R Square*	Std. Error of Est.	*R Square Change*	*F Change*	Df 1	Df 2	Sig. *F Change*
Allele	.09	.01	-.01	36.47	.01	0.38	1	44	.54
Allele, Age	.46	.21	.18	32.88	.20	11.13	1	43	<.01

Study Two results summary. Study Two investigated possible relationships between ToM and core ASD symptoms, and between ToM, age and 5-HTTLPR genotypes and alleles.

We hypothesized that ToM would correlate with measures of social communication and interaction, but this was not found. ToM scores did not correlate with any core ASD symptoms in this sample.

Age was assessed as a mediating factor for ToM ability. Age and ToM correlated strongly, and linear regression found that age accounted for 18.90% of the variability in ToM scores in this sample.

ToM scores were plotted against age for the total sample, as well as for each genotype, and no clear developmental patterns emerged. This suggested that the sample as a whole did not develop ToM in a similar way, and further that grouping by genotype did not reveal subgroups with difference patterns of development.

Multiple regression assessed the influence of genotype and age on ToM, and found that genotype contributed very little. This was also found for allele.

Study Two found no definitive evidence for 5-HTTLPR having a role in ToM development in ASD.

Discussion

This protocol was the first in a series of studies investigating possible biological mechanisms underlying deficits in social functioning in ASD. This protocol focused on the possible relationships between core ASD symptoms, social cognition in the form of Theory of Mind (ToM), and the genotypes of a candidate gene (serotonin transporter promoter length polymorphism, 5-HTTLPR). An increased prevalence of the short allele in the ASD group, as reported by Arieff et al. (2010), was confirmed in the current sample. No relationship between 5-HTTLPR and core ASD symptoms, between 5-HTTLPR and ToM, or between core ASD symptoms and ToM were found. The possible reasons for this are discussed below. The main limitation was the inadequate measurement of ASD characteristics that prevented the identification of accurate phenotypic data.

A main aim was to pilot ASD scales in a local sample. Relatively little research on ASD in South Africa is available in the literature, so the characteristics of the current sample and the performance of ASD scales within this sample were of interest. The Autism Social Skills Profile (ASSP), Social Communication Questionnaire-Lifetime (SCQ-Lifetime) and Repetitive Behaviour Scale – Revised (RBS-R) were used to assess impairment in the core symptom domains specified in the DSM. ASD is thought to present similarly across cultures, and scales should therefore perform similarly across these cultures. This protocol found that all three scales performed poorly in the current sample. All three scales had poor internal consistency and principal component factor analysis revealed latent structures not in keeping with the given subscales of each scale. Although the factor analyses results should be interpreted with caution due to the small sample size, these findings highlight significant problems using these scales in this way in a local sample. Future research should consider assisting parents when answering questionnaires as a poor understanding of ASD could have contributed significantly to the poor performance of these scales.

As accurate assessment of core ASD symptoms laid the foundation for this protocol, the use of these scales, and the general absence of sensitive measures for ASD research, is

discussed below. This is followed by a discussion of whether the symptoms assessed in this protocol support the DSM-5's merging of social communication and social interaction into a single symptom domain. Diagnostic criteria fundamentally inform our understanding of ASD, so investigating the integrity of this shift in diagnostic criteria is important when attempting to assess phenotypes.

The findings for Study One, which aimed to investigate possible relationships between core ASD symptoms and 5-HTTLPR, are discussed. Thereafter, Study Two, which investigated possible relationships between ToM and core ASD symptoms, and ToM and 5-HTTLPR, is discussed.

A South African ASD Sample

Research on ASD within South Africa is sparse. The population is diverse in race, culture, language, and socioeconomic status. This diversity must be considered as results from local studies may not be comparable to those of international studies with different demographics. Further, samples in South African research could represent a subgroup within the population, which would limit generalisability to the entire local ASD population. As the demographics of the local ASD population are not known, one must compare research samples to the demographics of the general population to establish if the sample is representative. Comparing the demographic data of local research samples to those of international samples is also a useful indicator of whether studies are comparable.

This protocol aimed to recruit a sample of children who not only reflected the variability seen along the ASD spectrum, but that included children from various backgrounds typical in the South African Western Cape ASD population.

DSM-IV-TR ASD subtypes. This protocol was designed in anticipation of the release of the DSM-5. As the DSM-IV-TR ASD subtypes were to be abandoned (and have since been), they were not used to guide recruitment. However, they were noted and used to assess whether the sample reflected the full ASD spectrum, or whether it instead reflected a subgroup within ASD. The full sample predominantly consisted of Autism diagnoses, followed by equal numbers of Aspergers and PDD-NOS diagnoses. IQ testing was not conducted, so high functioning and low functioning autism diagnoses were not identified, and were collectively grouped as Autism diagnoses. PDD-NOS and Aspergers were fairly well represented as each contributed just over 20.00% to the sample.

In this study all participants were recruited from Autism-specific special needs schools. PDD-NOS is a diagnosis which can often be made due to a shortage of adequate information to make a more specific diagnosis (Matson & Boisjoli, 2007; Prior et al., 1998). Being in an Autism-specific school makes it likely that the child has been formally diagnosed, and participation in this study required a formal diagnosis, so this likely explained the lower PDD-NOS numbers. Children with Aspergers typically struggle in mainstream schooling despite the absence of learning difficulties, and are therefore likely to be placed in Autism-specific schools. The high incidence of Aspergers was therefore also explained by sampling from these schools. All ASD subtypes were represented in this sample.

Sex. The total sample was predominantly male, with 6 female participants and 60 male participants. ASD is four times more prevent in males, so a male dominated sample was expected (Kogan et al., 2009). Although the recognised female to male ratio internationally is 1:4, our total sample showed a ratio of 1:10.5. The DSM-5 notes that within clinical samples, women with ASD tend to show comorbid intellectual disability, and those without intellectual disability or overt language delays may go unrecognised due to more subtle presentations (American Psychiatric Association, 2013). Female children with ASD who do not have intellectual impairment may have more subtle social and communication difficulties that are not as easily recognised, and are less likely to be diagnosed or placed in special needs schools than male children with ASD. Recruiting from Autism-specific schools could have increased the likelihood of males being recruited beyond the expected 1:4 ratio.

Inclusion of non-verbal participants. Many research studies cannot include non-verbal children in their protocols because these children are often unable to complete the necessary tasks. Examples of this are seen in studies that include Theory of Mind tasks: children are often asked to explain why someone did what they did, or what they were thinking at a specific time, so without speech a child cannot complete these tasks and will excluded from such studies (Happé, 1994; Scheeren et al., 2013). This leads to the risk that non-verbal children may be under-represented in the literature. This protocol included non-verbal children as far as possible. Parent-reports were used to collect data on core ASD symptoms, and DNA collection did not require verbal abilities from the child. Data from non-verbal children were therefore used in all analyses which did not involve ToM data – that is, the preliminary analyses and Study One. This allowed the inclusion of 12 non-verbal children. As this constitutes approximately 17.00% of the sample, the inclusion of these children was likely to contribute meaningfully in this protocol.

The inclusion of non-verbal children, the distribution of DSM-IV-TR ASD subtypes and the male to female ratio in the sample, indicate that the sample was diverse and did not represent a specific subgroup within ASD. The sample included children with a range of impairment, as is seen across the ASD spectrum. Demographic data was used to assess to what extent the ASD sample was representative of the South African Western Cape population.

Language. This sample was predominantly English speaking. This sampling bias was a direct result of the inclusion criteria used during recruitment: participants needed to be fluent in English or Afrikaans to undergo ToM testing, and their parents needed to be fluent in English to complete the symptom scales. Ideally the scales would be translated into all local languages, and testing would be conducted via trained interpreters in any local language so that recruitment would not be limited, but this was not feasible within the limited scope of a Masters research project. Moreover, as this project laid the foundation for later, larger phenotype-genotype studies in ASD, the current study aimed to assess usefulness and appropriateness of these scales in English speakers before proceeding to translation. The current sample was thus limited.

SES was almost equally distributed across the low, medium and high strata according to an SES algorithm adapted to better identify differences in low SES communities. As mentioned in the Results section, this protocol did not use traditional markers of SES. Instead, SES was calculated using an algorithm that considered estimated household income, highest level of education for parents, and an asset index (Myer et al., 2004). This algorithm allows better differentiation of SES in areas with lower income, but it can over-estimate the wealth of those in middle to high SES strata. Therefore, although this algorithm found that the current sample included children from all socioeconomic backgrounds, those in the middle and high strata might not be ranked as such with traditional SES markers. Traditional markers would likely find a higher incidence of low SES, and a lower incidence of high SES in this sample.

Race. There were equal numbers of coloured and white participants, which together accounted for approximately 75% of the sample. The literature tends to report findings from studies in Western countries (US, UK and Europe), which usually have predominantly white samples. The racial and cultural diversity found in South Africa makes it a unique environment for psychological research, and also makes it more important that demographics are considered when comparing local findings to those reported in the literature. Our sample included participants from different racial groups, although we had increased rates of white participants and decreased rates of black participants.

Unfortunately, due to the political history of South Africa there remains socioeconomic inequality across race, and children from lower socioeconomic environments are less likely to be diagnosed and/or to receive appropriate care (Bertrand et al., 2001; Fombonne, 2003; Malcolm-Smith et al., 2013). Races from low SES communities are likely to be under diagnosed and underrepresented in research, which could explain our lower rate of black participants, as they are less likely to be correctly diagnosed and placed in special needs schools where the current research was conducted. The parents of these children are also less likely to be fluent in English (an inclusion criteria for this protocol), and were therefore less likely to be recruited. Therefore, although different races were represented in the sample, black children were underrepresented and this limits the generalisability of any findings.

Race and 5HTTLPR. Race also needs to be considered when comparing the genotyping data from our study with that of previous local studies on 5-HTTLPR. As mentioned previously, two studies have explored the distribution of 5-HTTLPR in South Africa. The first was a study of 109 individuals with ASD from Cape Town and Gauteng in 2010 (Arieff et al., 2010), and the second assessed the distribution of 5-HTTLPR in the general South African population in a sample of 342 individuals (Esau et al., 2008). Our sample showed a much higher percentage of *l/s* genotypes, a lower percentage of *s/s* genotypes, and a much lower percentage of *l/l* genotypes compared to the other local ASD study. We note that despite the differences in our samples and that of Arieff et al.'s (2010) ASD sample, both showed a considerably higher rate of the short allele than the local non-ASD sample. A possible explanation for the differences in allelic distributions between our sample and that of Arieff et al.'s (2010) was that our sample was limited to the Western Cape, whereas Arieff et al.'s included Gauteng.

A comparison with the Arieff et al. (2010) ASD sample found their study had similar rates of white participants, a higher rate of black participants, and a lower rate of coloured, mixed and "other" participants. Arieff et al.'s (2010) study only listed three racial groups (i.e. white, black and coloured) whereas we listed five groups (i.e. white, black, coloured, Indian, and "other"). Individuals who listed themselves as Indian, coloured, or "other" may have very different genetic heritage, which could explain the disparity in genotype distribution between the current study and Arieff et al.'s (2010) study. Esau et al.'s (2008) article explored the distribution of 5-HTTLPR across various populations globally and found that local black and coloured populations showed significant differences in allelic distribution to most other populations globally and that genetic heritage influences 5-HTTLPR allelic distribution. For a

full discussion of 5-HTTLPR distributions across different global populations see Esau et al. (2008).

The current sample had a different racial distribution to those of the two previous studies which explored the genotype and allelic distribution of 5-HTTLPR in South African samples. Using race as an indication of genetic heritage, these differences in racial distribution could explain the differences in 5-HTTLPR distribution across samples. Ideally studies would recruit sufficient participants from each racial group to ensure that the distribution of 5-HTTLPR within that race is represented. However, as is discussed under Limitations, this would require a very large sample size and would not be feasible within the time and resource constraints of a Masters study, or within most studies recruiting from a limited clinical population.

Verbal ability and 5-HTTLPR. A change in allelic distribution for this sample was also noted across Study One and Study Two, suggesting it changed when non-verbal children were excluded. This is discussed later, but is mentioned here as it may be a further explanation for the differences in 5-HTTLPR distributions between the current sample and those of the two previous studies on 5-HTTLPR conducted locally. Unfortunately those two studies do not provide information on the verbal ability of their participants, so one cannot establish if this influenced their recruitment. However, it is likely that differences in racial distributions and level of inclusion of non-verbal children collectively contributed to the differences in 5-HTTLPR distributions noted between the current protocol and those of the Arieff et al. (2010) and Esau et al. (2008) studies.

Generalisability of findings based on characteristics of the current sample. Our sample included children from across the ASD spectrum, with various DSM-IV-TR ASD subtype diagnoses and a range of verbal abilities. These children were from various races and SES backgrounds. The sample was somewhat homogenous regarding home language and sex ratios as it predominantly included first-language English speakers and male children. However, comparisons between the demographic data of the current study and those of previous studies on 5-HTTLPR in the general and ASD local populations suggest that the sample included children from various subgroups seen in the ASD population in the Western Cape. Providing detailed demographic data for the current sample should allow other researchers to compare the characteristics of their samples to the current sample, which will then inform whether results from this protocol will be comparable to results from other ASD studies.

Preliminary Analyses: ASD Diagnostic Criteria and the Scales Used to Assess These Symptoms

The ASSP, SCQ-Lifetime and RBS-R were used to assess core ASD symptoms in this protocol. The psychometric properties of each scale were assessed, and the latent structure of each scale was explored. The limitations of these scales are discussed below, followed by discussion regarding how findings of these analyses support the shift in diagnostic criteria in the DSM-5 to merge social communication and social interaction into a single symptom domain. This study aimed to assess relationships between core ASD symptoms, ToM and 5-HTTLPR, and as current diagnostic criteria inform how we conceptualise core ASD symptoms, it was essential that this shift in diagnostic criteria was assessed.

Performance of the ASD scales. Three scales were used to assess core ASD symptoms, with each scale selected to focus mainly on one of the core symptoms domains (i.e. the ASSP for social interaction, the SCQ-Lifetime for social communication, and the RBS-R for restricted and repetitive behaviours and interests). Cronbach's alpha and principal component factor analysis explored the psychometric properties and latent structures of these scales. These scales performed poorly and had to be adjusted to preserve, at least in part, the integrity of the data. Principal component factor analysis was used extensively to explore the structure of these scales. However, it is generally accepted that 10-15 participants are recruited per variable when one performs factor analysis. The nature of this research, namely that it was conducted in a limited clinical sample within the time and cost limitations of a Masters thesis, made recruiting such a large sample unfeasible. All findings based on principal component factor analysis therefore need to be interpreted with caution, and ideally the performance of these scales should be reassessed with a larger sample.

Social communication and social interaction. The DSM-IV-TR conceptualized social interaction and social communication as independent of one another (American Psychiatric Association, 2000). The DSM-5 recently updated the ASD diagnostic criteria and merged these two symptom domains into a single dimension for social communication and interaction. This protocol was initiated prior to the release of the DSM-5, but in anticipation thereof. It therefore aimed to measure social communication and social interaction independently, so as to align with the DSM-IV-TR, but with the possibility of merging them into a single dimension when the DSM-5 was released. This also provided an opportunity to assess whether the two symptom domains were truly independent of one another, or whether they should be merged.

The ASSP and SCQ-Lifetime were used to measure social interaction and social communication respectively. The ASSP predominantly focuses on social skills related to social interaction, while the SCQ-Lifetime focuses on social communication abilities (Bellini & Hopf, 2007; Rutter et al., 2003). Although each scale claims to have a specific focus (i.e. social interaction versus social communication), their subscales and assessment of the included items show that each scale has a significant overlap between these two symptom domains. However, individual questions either assess social interaction or social communication. Using scores for questions, rather than for subscales, allowed us to assess whether these symptom domains could be disentangled, or whether they should be conceptualised as a single symptom domain.

The ASSP has increased sensitivity for detecting differences in social interaction (and, to some degree, social communication) as it was designed to track improvement when a child enters an intervention procedure. However, the ASSP is not widely used in research. We therefore used the SCQ-Lifetime to support these scores, with the intention that the SCQ-Lifetime subscale for "Social Interaction" would correlate well with ASSP scores. Although the SCQ-Lifetime is not as sensitive as the ASSP, it is widely recognised in ASD research.

The literature reported sound psychometric properties for both scales. As ASD is expected to present similarly across cultures, the very poor performance of these scales in this protocol was not anticipated. Analyses with Cronbach's alpha revealed poor internal consistency for both scales and principal component factor analyses did not support the given subscales for either scale. This poor performance became apparent during data analysis, and it was therefore decided to try protect the integrity of the data through statistical adjustments. This required the removal of all weak items, abandoning given subscales and instead establishing composite scores based on principal component factor analysis.

Social interaction and the Autism Social Skills Profile (Bellini & Hopf, 2007). The ASSP was designed to provide a comprehensive measure of social interaction ability. The number of measures available to identify and assess impairment in specific social skills within an ASD sample is limited. In light of this, Bellini and Hopf (2007) developed the ASSP. This measure provides a total score for social ability, and then scores these behaviours on three subscales: Social Reciprocity; Social Participation/Avoidance; and Detrimental Social Behaviours.

The ASSP is a relatively new measure, and has not yet been widely used in research. It was designed for use in intervention planning to identify the specific social deficits that need focused intervention. The use of a four-point likert scale allows the ASSP to track progress

once an individual has entered, or completed, an intervention. Scores are thus intended to be sensitive to different levels of skills and areas of impairment.

The ASSP was chosen for use in this protocol because it measures a sufficiently diverse range of social skills and is argued to be sensitive to differences in degree of impairment in social interaction. This protocol included children from across the ASD spectrum, with some participants being non-verbal while others were considered to be high functioning. However, all participants had some degree of impairment in social interaction as this is a requirement of the ASD diagnosis. A scale was needed that would detect this impairment in all cases, and be sensitive enough to distinguish the level of impairment across these cases.

Bellini and Hopf (2007) reported high test-retest reliability and internal consistency, but this was not replicated in this protocol. Using Cronbach's alpha to assess internal consistency, we found problematic alpha values for the scale as a whole, for subscales, and that twelve of the forty-nine questions had a weak correlation to the total score (Field, 2009); this suggests that they are not measuring what the scale set out to assess. These items were removed. The latent structure of the ASSP was then explored with principal component factor analysis with varimax rotation, and a three factor solution that reflected the given subscales mentioned above did not emerge (Appendix H).

Models in line with either the DSM-IV-TR or DSM-5 were then considered. The latent structure revealed that a single factor model was statistically best supported. Further, this model was theoretically sound as other models showed arbitrary loadings (i.e. separated items that assess the same symptom domain, or grouped items that assess different symptom domains). Twenty-six of the retained items loaded moderately to strongly (i.e. $r > .50$) onto a single factor model. To ensure scores were a truer reflection of ability in the child, we only used these twenty-six questions to calculate a composite score. Analysis of the retained questions revealed that they included items that measured social interaction and items that measured social communication, so the composite score was used as a measure of Social Communication and Interaction (ASSP) as a single symptom domain. The meaning of this in regards to support for or against the DSM-5 diagnostic criteria is discussed later.

Social communication and the Social Communication Questionnaire - Lifetime (Rutter et al., 2003). The SCQ-Lifetime was designed to assess a range of social communication abilities within an ASD sample (Berument et al., 1999). The SCQ-Lifetime contains 40 Yes/No items that assess social communication ability across the lifetime, but with a focus on the child's communication ability between 4 and 5 years old (Berument et al., 1999). This

measure is reportedly sensitive to different levels of impairment in social communication (Berument et al., 1999). It was designed based on the Autism Diagnostic Interview-Revised, which is a standardised semi-structured interview that provides an algorithm for ASD diagnoses (Lord, Rutter, & Le Couteur, 1994). The questions in the SCQ-Lifetime correspond to the DSM-IV ASD diagnostic criteria, with four subscales: Communication; Social Interaction; Stereotyped Behaviours; and Abnormal Language. The Communication and Abnormal language subscales would have been of interest to assess social communication, while the other two subscales would have been used as collateral to support scores from the ASSP and RBS. However, analysis of the SCQ's performance in this sample did not support the use of these subscales.

SCQ-Lifetime was chosen because it is frequently used in ASD research and has well established validity and reliability (Berument et al., 1999; Eaves et al., 2006; Witwer & Lecavalier, 2007). A further benefit was hoped for in the use of the SCQ-Lifetime Social Interaction subscale to provide support for the ASSP's assessment of social interaction. As the ASSP is not as frequently used in research as the SCQ, we had hoped that agreement in ASSP and SCQ-Lifetime Social Interaction scores would indicate that results were valid. The Stereotyped Behaviours subscale could serve as collateral to RBS-R scores. However, as the subscales were not supported in our analysis, this was not possible.

Despite the SCQ-Lifetime having well established validity and reliability, this scale performed poorly in the current sample. The internal consistency was low for the scale as a whole, and indicated fourteen of the forty questions had a weak correlation to the total score. This indicated that these twelve questions measured something different to what the scale as a whole was assessing (Field, 2009). These items were considered weak and were removed. These items are discussed later, but it is noted here that they did not assess the same symptom domain, but were items from each of the core ASD symptom domains. The subscales were not supported by principal component factor analysis (Appendix J).

The SCQ-Lifetime assesses various ASD characteristics, and includes items from all three symptom domains in the DSM-IV-TR (i.e. social communication, social interaction, and restricted and repetitive behaviours and interests). The retained items still reflected all three symptom domains. It was expected that further statistical analyses would isolate the questions relating to restricted and repetitive behaviours and interests as this symptom domain is independent from that of social communication and interaction. This factor could then be used to support scores from the RBS-R. Statistical analysis would also indicate whether social

interaction and social communication could be measured independently using items from this scale.

Principal component factor analysis with varimax rotation was run on the retained questions. Several models were considered, but all models showed arbitrary splitting of items such that items that clearly measured the same aspect of ASD were split across factors, while items that were clearly unrelated would load onto the same factor. Statistical support for these models was moderate at best, and the absence of a coherent theoretical framework to support them meant these models could not be used as each factor did not logically represent a cohesive aspect of ASD.

The retained questions were re-assessed, and based on the current understanding that social communication and interaction is independent from the symptom domain of restricted and repetitive behaviours and interests (American Psychiatric Association, 2013), all items assessing restricted and repetitive behaviours and interests were removed. Principal component factor analysis was rerun on the remaining nineteen items to assess whether a two factor model (i.e. one that separated social communication from social interaction as in the DSM-IV-TR) or a single factor model (i.e. where social communication and interaction represented a single symptom domain as in the DSM-5) would be better supported. The latent structure revealed that a single factor model was statistically best supported, and this model was theoretically sound. Twelve of the retained items loaded moderately to strongly (i.e. $r >$ .50) onto a single factor model. To ensure scores were a truer reflection of ability in the child, we only used these twelve questions to calculate a score for Social Communication and Interaction (SCQ). As the SCQ includes 40 items, the number of items that were excluded was worrying.

As with the ASSP, questions relating to social communication and those relating to social interaction could not be separated statistically, and were merged. The implications for this in regards to support for or against the DSM-5 ASD diagnostic criteria are discussed later.

Restricted and repetitive behaviours and interests. The RBS-R was used to assess restricted behaviours and interests. Both the DSM-IV and DSM-5 identify this as an independent symptom domain, and include impairment in this domain as necessary for an ASD diagnosis.

The RBS-R was selected for use in this protocol because it measures a sufficient range of restricted and repetitive behaviours and interests seen in ASD and was designed for use within ASD samples. The RBS-R contains 43 questions which are answered on a four-point likert scale from “does not occur” to “severe presentation”. This range of restricted and

repetitive behaviours and interests is scored on five subscales: Restricted Interests; Ritualistic/Sameness Behaviour; Stereotyped Behaviours; Compulsive Behaviours; and Self-Injurious Behaviours.

The RBS-R has shown acceptable reliability ratings (Lam & Aman, 2007), but this was not replicated in the current protocol. The internal consistency analysis was low for the scales as a whole and did not support the given subscale structure. It also found that 4 of the 43 questions correlated poorly to the total scores and these were thus eliminated (Field, 2009) as this suggests that these four questions assessed something other than what the scale aimed to assess. Exploration of the latent structure of the RBS-R with principal component factor analysis with varimax rotation did not find a model that reflected the listed sub-scales.

The DSM-IV-TR and the DSM-5 both list restricted and repetitive behaviours and interests as a single symptom domain, so a single factor model was assessed. The scree plot suggested that a two factor model was also likely. As discussed in the Literature Review, it is possible for a child to present with restricted interests without the motor elements of this symptom domain – that is, they may not show restricted or repetitive physical behaviours, but will have a certain topic they repeatedly talk about, read about, and generally focus on. We therefore assessed a two factor model in case items assessing restricted interests were separate from those assessing repetitive motor behaviours.

A single factor model was found to be best supported. Principal component factor analysis with varimax rotation showed that 27 of the remaining 39 items loaded moderately to strongly (i.e. $r > .50$) onto the single component. These items were used to calculate a Restricted and Repetitive Behaviours and Interests (RBS-R) score for each participant.

The retained questions reflected a range of characteristics included in the DSM-5 symptom domain of restricted and repetitive behaviours. As the final ASSP and SCQ-Lifetime models did not include items reflecting impairment in this domain, the composite scores calculated for the RBS-R were the only measure of impairment in restricted and repetitive behaviours and interests used in this protocol.

Limitations of ASD scales. The ASSP, SCQ, and RBS-R all showed poor psychometric performances within the current sample. This is discussed, with a focus on the characteristics of the local population that could undermine the performance of these scales. This is followed by a brief discussion of more general concerns regarding these scales.

Poor psychometric performance in the current sample. The SCQ-Lifetime and RBS-R are frequently used in research, while the ASSP's use to date is limited. The literature reports acceptable reliability ratings for all three scales. However, when each scale was

assessed in this protocol, none were found to be psychometrically sound in their original form.

Internal consistency was problematic across all scales. Cronbach's alpha identified several weak items in each scale. These questions had to be removed to protect, to some extent, the integrity of the data. However, when the removed items were assessed, it was noted that these included questions relating to certain common and important characteristics seen in ASD. For example, the SCQ-Lifetime question "Has she/he ever had any mannerism or add ways of moving his/her hands or fingers, such as flapping or moving her/his fingers in front of her/his eyes?" and the RBS-R question "HAND/Finger (flaps hands, wiggles or flicks fingers, claps hands, waves or shakes hand or arm)" were both removed. Flapping can be very common in ASD, and may be regarded as a key sign for ASD, yet questions measuring this behaviour did not perform well within these scales for this sample.

Some of the items that were removed are generally recognised as key ASD characteristics in ASD diagnostics and interventions. This is problematic as it indicates that the very measures used to identify key aspects of ASD may not do so particularly well. As mentioned before, each symptom domain in ASD includes a diverse range of behaviours or abilities. Scales cannot be designed with high specificity if our conceptualisation of ASD does not include the identification of necessary key symptoms, or when impairment in a certain symptom domain cannot be identified unless several areas are assessed. The range of possible signs to indicate core deficits could therefore contribute to the poor performance of ASD scales.

In addition, factor analysis indicated that none of the scales possessed the latent structures supposed to be present in the form of their respective subscales. Further assessment of the latent structures of the measures revealed that theoretically unrelated questions loaded onto the same factors, while questions which were inseparable in theory loaded onto different factors. Extensive adjustments had to be made to each scale to overcome redundant questions and to identify latent structures that were both statistically and theoretically sound. However, even with these adjustments, the selected strongest models showed many weak loadings. Statistical correction could not compensate for the poor performance of these scales, and although the data was protected to some extent, concerns regarding these scales and scores derived from them remain.

Considerations of the local population in regards to the performance of these scales. This protocol recruited children with confirmed ASD diagnoses, and these children were relatively diverse in race, socioeconomic status, in verbal functioning, and DSM-IV-TR ASD

subtypes were represented. As these measures were all designed for use in ASD samples they should have remained somewhat sensitive to differences in core ASD symptoms in any ASD sample, regardless of its diversity. The poor performance of these measures was therefore unexpected. In an attempt to explain this poor performance, the studies which assessed the psychometric properties of the ASSP, SCQ, and RBS-R were assessed.

The reported validity and reliability statistics for these scales were from samples in the United Kingdom and United States of America, so it is possible that the differences between these samples and the diverse local South African population undermined the use of these scales locally. Bellini and Hopf (2007) assessed the ASSP in a predominantly white, English speaking sample. The current sample was predominantly English speaking as well, but almost a third of the sample was from a low SES background and the racial distribution across our sample was more diverse. As with the ASSP, the psychometric properties of the SCQ-Lifetime were also established in a predominantly white, English-speaking sample that is not comparable to the local South African population.

The local population is very different to those of the United Kingdom and United States of America in culture, race, SES, and education. Questions designed for use in those populations may not be culturally appropriate for local use due to differences in languages, references that are unfamiliar to South Africans, or differences in cultural norms. For instance, the SCQ question "has she/he ever used socially inappropriate questions or statements? For example, has she/he ever regularly asked personal questions or made personal comments at awkward times?" can be understood very differently across cultures.

Further, low SES parents in South Africa may have poor education that undermines their ability to read and understand the questions on these scales. They may misunderstand questions or the instructions, and their answers may not be a true reflection of that aspect of their child's ASD presentation. A large screening study conducted by the University of Cape Town's Autism Research Group found that even when scales are translated into the parent's home language, they struggle to answer questions without additional assistance and explanations from ASD experts (Bozalek & Malcolm-Smith, 2014). Previous studies noted that scales performed better in samples of parents whose children had formal ASD diagnoses (Chandler et al., 2007), but this was not true for the current study. As is discussed, this could be due to the demographics of the current sample.

As mentioned before, there is the additional problem of poor resource availability for families dealing with ASD in South Africa. Parents may have very little education regarding ASD, and this contributes to a poor understanding of questions, as well as a limited ability to

recognise the necessary behaviours and characteristics in their children. The validity of their answers may therefore be undermined.

This study was unable to assess whether the abovementioned aspects (i.e. culture specific questions, parents' education, and parents' understanding of ASD) were the reasons for the poor performance of the ASSP, SCQ, and RBS-R within this sample, but the possibility that they had an impact is recognised.

Other concerns and challenges regarding the ASD scales. In addition to the poor psychometric properties found in these measures, we note concerns regarding their sensitivity, the comparability of the scales, and the use of parent reports.

The sensitivity of the ASD scales. The SCQ-Lifetime only assesses the presence (or absence) of characteristics, and not the severity of these characteristics. Individuals with slight, moderate, or severe impairment in a certain area will all score as "impairment present", meaning they will all score equally. For example, a child who may bite himself when anxious, but only does so once every few weeks, and a child who bangs his head daily would both answer "yes" to the question "has she/he ever injured her/himself deliberately, such as by biting his/her arm or banging her/his head?" and the difference in severity between these cases will be lost. The SCQ-Lifetime is therefore unable to differentiate the level of impairment across these cases.

The ASSP and RBS-R should have been more sensitive to these differences as they measured the degree of impairment across characteristics rather than their mere presence. However, as scores were summed, this comes with the risk that individuals with slight to moderate impairment in multiple areas would have similar total scores to individuals with severe impairment in fewer areas. Subscales could have been used to indicate whether impairment was high in a specific area or moderate across several areas, but as the focus of this protocol was on core ASD symptom domains and not the ASD characteristics measured by subscales (unless they align with a core symptom domain, as is seen with the SCQ-Lifetime subscales), the subscales were of little help. Further, the subscales were not reliable in this sample.

The comparability of the scales. Although the ASSP and SCQ-Lifetime were found to measure the same symptom domain, we found only a moderate correlation between these scores. This is likely due to the different time periods each scale focuses on (i.e. current ability versus ability across the lifespan but with a focus on when the child was 5 years old) and the forms of measurement (i.e. Yes/No compared to a four-point likert scale). Focusing on different periods in a child's development results in scores for a past presentation (SCQ)

and for a current presentation (ASSP), and one cannot assume that the ASD presentation has been consistent across this time, especially with older participants who have had more time to develop coping strategies or undergo interventions. Further, as was mentioned above, measuring the presence of a characteristic is not an indication of the severity thereof and thus cannot be compared to scores that do represent level of impairment.

The limitation of parent reports. These measures are all based on parent reports. Parents complete the questionnaires on their own, in their own time. The benefit of this approach is that larger amounts of data can be collected in a shorter period and without the need to employ clinicians. However, this comes with risk to the quality of the data. As discussed above, parents may misunderstand the questions on these measures. This could be due to language barriers, literacy levels, socio-economic status, or a limited understanding of ASD and its associated characteristics.

The current sample included many participants from low SES backgrounds that may have poor literacy. Insufficient resource availability to provide support for families dealing with ASD means one cannot assume that the parents of a child with a formal ASD diagnosis have been educated on this disorder, especially in families from a low SES background. We therefore recognise that parent reports were not the ideal format for data collection in the current sample. These limitations should be considered when designing future ASD measures.

Considerations for future ASD scales. One of the greatest challenges in research and in bettering our understanding of this disorder is the heterogeneous nature of ASD presentations. Establishing clear, specific phenotypes is essential in ASD research. Ultimately all studies should aim to establish very clear phenotypes for participants. This would require establishing detailed, extensive symptom profiles for each participant that would then allow researchers to identify both similarities and differences between participants. However, ASD presentations are so varied, with such a range of possible behaviours and deficits, and a range of level of impairment in each area, that this is a very challenging task. It is the core symptoms of deficits in social communication and interaction and restricted and repetitive behaviours and interests that represent the basis of this disorder.

As mentioned above, scales with scores that indicate degree of impairment are unable to differentiate between children with severe impairment in a specific area compared to moderate impairment across several areas. One way to differentiate these cases would be to identify the main presentations within each domain (for instance, that restricted interests could occur without repetitive physical behaviours) and develop subscales that validly and reliably measure impairment in each of these areas. The current scales have attempted this,

but the validity and reliability of subscales remains controversial (Bellini & Hopf, 2007; Gau et al., 2011; Lam & Aman, 2007; Mirenda et al., 2010; Witwer & Lecavalier, 2007), and the current study did not support the use of these subscales. It is possible then that these scales may not be able to identify subtle differences in impairment across cases.

Further, to prevent loss of quality of data due to parents misunderstanding questions, researchers can either educate parents on ASD or be present and conduct interviews with the parents instead of having them complete the scales on their own. Questions may need to be phrased differently across cultures where behaviours are understood differently. Having a researcher or clinician present would ensure that parents understood the questions, that questions were relevant, and the researcher could use their knowledge to ensure that answers provided by the parents were valid. This would give the researcher the opportunity to query parents' answers and gain more detail on the child's presentation.

Ideally one could also supplement these scales with the researchers' qualitative impressions from their interactions with the children. The ADOS is a good tool for getting a better understanding of a child's presentation, as is the use of the ADI-R, but using these tools requires a great deal of time and the use of clinicians with a specific skill set, which increases the cost of the research.

The local population is very different from those of the United Kingdom and the United States of America, so all tools should be piloted and adapted for local use. The current protocol laid the foundation for future genotype-phenotype studies, and therefore served as a pilot for these scales within the local ASD population. All three scales performed poorly in the current sample. However, the ASSP and RBS-R may be useful if adapted for the local context. The SCQ's performance was so poor that there is little evidence in this protocol to support using it in local ASD research. The literacy and SES of local participants must always be considered, and in many cases it may not be ideal to rely heavily on parent reports. In order to establish clear phenotypes for local children with ASD, then, it is important that focused, refined scales are developed, and that these are then administered in a way that ensures parents fully understand the questions. Establishing clearer phenotypes may be less cost and time efficient than using existing parent report scales, but it is necessary – research into genotype-phenotype relationships cannot make progress unless clear phenotypes are established. This protocol found that assessing only core ASD symptoms may be too blunt an approach as each domain includes such a broad range of signs and symptoms. A more refined approach is needed.

The development of better scales to assess ASD characteristics, and our understanding of ASD as indicated by current diagnostic criteria are fundamentally intertwined. The limitations of current ASD scales impact assessment and research in ASD. This then limits the progress necessary to further refine diagnostic criteria. The current protocol assessed whether the merging of social communication and social interaction by the DSM-5 was supported. This was necessary as it informed how the current protocol conceptualised core ASD symptoms and how it then measured these symptoms.

Support for the DSM-5 diagnostic criteria. As this protocol was designed prior to the release of the DSM-5, it was designed to assess social communication and social interaction independently. This provided the opportunity to assess the integrity of the shift in diagnostic criteria from the DSM-IV to DSM-5.

Social communication and social interaction. The ASSP was used to mainly assess social interaction and the SCQ-Lifetime to assess social communication. However, each scale did include some questions that assess the other domain to some extent (i.e. the ASSP has some questions that assess aspects of social communication, and the SCQ-Lifetime has a social interaction subscale).

Statistical analyses of each scale was used to see whether items which assess social interaction would load together, but be independent of those that assessed social communication, and vice versa. However, instead it found that both scales were best represented by models that combined these two domains. Exploratory principal component factor analysis of both measures showed that questions assessing social communication could not be disentangled from questions assessing social interaction. This is line with previous research (Boomsma et al., 2008; Frazier et al., 2008; Mandy et al., 2012; Snow et al., 2009; van Lang et al., 2006)**,** suggesting that the separation of these domains according to the DSM-IV-TR was arbitrary, and that the DSM-5 diagnostic criteria may be a truer reflection of core aspects of ASD.

Restricted and repetitive behaviours and interests. Although the DSM-5 diagnostic criteria stipulate that individuals with ASD must have impairment in both core ASD symptom domains, these domains are still considered to be relatively independent of one another. As all participants had received formal diagnoses of ASD we expected a slight correlation between social communication and interaction scores and restricted and repetitive behaviours and interests scores. This was true, as Restricted and Repetitive Behaviours and Interests (RBS-R) scores correlated with both the ASSP and SCQ-Lifetime scores for Social Communication and Interaction. The correlation was highest between composite scores from the RBS-R and

ASSP. As both these scales use four-point likert scales to assess level of impairment, they have increased sensitivity compared to the SCQ-Lifetime (which assesses only the presence or absence of impairment). The level of measures in each scale may explain why the ASSP could have better correlated with the RBS-R than with the SCQ-Lifetime.

This protocol used the ASSP, SCQ-Lifetime, and RBS-R to assess core ASD symptoms and plot them meaningfully onto core symptom domains. The current protocol found that social communication and social interaction were inseparable, and that restricted and repetitive behaviours and interests had to be a separate symptom domain. Together these analyses support the DSM-5 diagnostic criteria which model social interaction and social communication as a single symptom domain, and restricted and repetitive behaviours and interests as a second, independent, symptom domain.

Preliminary analyses conclusion. The preliminary analyses in this protocol assessed the psychometric properties of ASD scales used to assess core ASD symptoms and then explored whether these data supported the recent DSM-5 ASD diagnostic criteria.

Data on the performance of ASD scales in South Africa are limited. It was therefore important to ascertain whether the scales could be used locally. The current protocol is the first study within a larger study on the biological basis for social deficits in ASD, and one of the main aims was to assess the usefulness of current ASD symptom scales in establishing different phenotypes by piloting them in a local ASD sample.

Statistical analyses with Cronbach's alpha and principal component factor analyses revealed that all three scales had poor internal consistency, included weak items, and had latent structures that were not representative of the standard subscales.

Statistical analyses were used to attempt to protect the integrity of the data from these limitations. Weak items were removed and the latent structure of each scale was assessed. This statistical investigation was informed by current theoretical conceptualizations of the structure of ASD symptoms, and composite scores were calculated for each participant for each scale. These scores, however, were undermined by the shortcomings of the scales. Each score included a board number of items included in that core symptom domain, yet certain key ASD characteristics had been excluded due to poor Cronbach's alpha scores. The composite scores were necessary to preserve the integrity of the data to some extent, but they were not ideal.

These analyses found that questions assessing social communication and questions assessing social interaction could not be disentangled, but that those assessing restricted and

repetitive behaviours and interests were separate. This preliminary analysis therefore supported the DSM-5 diagnostic criteria over those of the DSM-IV-TR.

The scores calculated from these scales during the preliminary analyses, namely Social Communication and Interaction (ASSP), Social Communication and Interaction (SCQ-Lifetime), and Restricted and Repetitive Behaviours and Interests (RBS-R) assessed core ASD symptoms within this sample. These scores were used alongside genotyping data and ToM scores to test the hypotheses laid out at the start of this protocol. The findings from Study One are discussed below, followed by those of Study Two.

Study One: ASD Phenotypes for 5-HTTLPR

Study One explored whether relationships existed between 5-HTTLPR and the core symptom domains of ASD, namely social communication and interaction and restricted and repetitive behaviours and interests.

Social communication and interaction. Social communication and interaction were assessed by the ASSP and SCQ-Lifetime. Social Communication and Interaction (SCQ-Lifetime) scores did not differ across genotypes or alleles. In hindsight, it seems clear that as the SCQ-Lifetime scores only indicated presence or absence of impairment in this domain, and as impairment is required for an ASD diagnosis, individual differences were unlikely to emerge. Social Communication and Interaction (ASSP) scores, however, were more sensitive to differences in impairment than Social Communication and Interaction (SCQ-Lifetime) as the ASSP was designed to measure levels of impairment within an ASD sample, and to track differences within individuals as they complete interventions. Neither genotypes nor alleles showed an effect on these scores either. Between-group differences across genotypes were marginally larger for Social Communication and Interaction (ASSP) than they were for Social Communication and Interaction (SCQ-Lifetime) scores, but were still negligible. Neither of the scores for impairment in social communication and interaction changed across 5-HTTLPR genotypes or alleles.

Tordjman et al. (2001) grouped participants into normal transcription and reduced transcription groups and reported that 5-HTTLPR alleles affected overall ASD impairment in social communication and interaction within a sample of 71 French participants with ASD. They used the ADI-R to assess impairment in this domain, and found those with reduced serotonergic transmission (i.e. the *l/s* genotype and *s/s* genotype) had greater impairment. The ADI-R is more extensive than the SCQ-Lifetime and the ASSP, and is an interview of the caregiver conducted by a clinician whereas the SCQ-Lifetime and ASSP are completed by a

caregiver alone. However, the SCQ-Lifetime was designed from the ADI-R and these are therefore comparable measures. Tordjman et al.'s (2001) study may arguably have had greater sensitivity than the current protocol, but results should have been comparable. The current study did not replicate the Tordjman et al. (2001) finding that reduced serotonergic transmission as indicated by 5-HTTLPR allele was related to greater overall impairment in social communication and interaction.

Brune et al. (2006) used the ADI-R and ADOS to assess core ASD symptoms across genotypes in 73 participants with ASD. Their study did not replicate the Tordjman et al. (2001) finding that overall ADI-R score varied across genotype, which is in keeping with the current absence of finding for overall ASSP and SCQ-Lifetime composite scores. By assessing specific items from the scales they found that specific characteristic were affected by 5-HTTLPR. They found participants with a short allele had greater impairment in the ADI-R domain of "failure to use nonverbal communication to regulate social interaction" while those with long alleles showed deficits directing facial expressions as assessed by the ADOS. These two characteristics would both fall under the social communication and interaction diagnostic criteria of the DSM-5. Brune et al. (2006) were able to measure core ASD symptoms with a greater sensitivity than the current protocol. Although our scores were refined for greater sensitivity and to overcome psychometric weakness in each measure, the composite scores remain less sensitive than specific scores from the ADI-R and ADOS.

Evidence for 5-HTTLPR influencing social communication and interaction in ASD remains unclear. Findings in the current study were undermined by poor measures used to assess core ASD symptoms, and existing literature lacks sufficient data on their participants for clear phenotypes to be established. To my knowledge the current study is only the third to attempt to relate ASD symptoms and characteristics to 5-HTTLPR genotypes and/or alleles. It is not possible to draw final conclusions when so little research has been conducted in this area, but the findings of this study suggest that 5-HTTLPR does not influence social communication and interaction within ASD.

Restricted and repetitive behaviours and interests. Brune et al. (2006) found that participants with the *l/l* genotype displayed greater deficits in "unusual sensory interests" on the ADOS, and aggression and stereotyped and repetitive motor mannerisms on the ADI-R, compared to the children with short alleles. This suggested that the group with normal serotonergic transmission showed greater deficits in the restricted and repetitive behaviours and interests symptom domain.

The current protocol assessed overall rate of impairment in Restricted and Repetitive Behaviours and Interests (RBS), but did not find any difference in scores across genotypes or alleles. As with social interaction and communication, this exploration was limited by the bluntness of the measure used. As subscales were not reliable, and principal component factor analysis did not reveal meaningful clusters of items to form new subscales, we could only use total scores and could not identify discrete areas of impairment within this symptom domain.

Brune et al.'s (2006) finding that participants with normal serotonergic transmission (i.e. the *l/l* genotype) exhibit greater impairment seems counterintuitive. Reduced or excessive serotonergic transmission could be considered clinically significant, but normal transmission is not. As such, if 5-HTTLPR is implicated in impairment in an area then it would have to be due to it undermining serotonergic transmission. The Brune et al. (2006) finding is therefore not logically sound, and it is likely that another factor that was not accounted for could explain the finding.

It is worth noting, however, that 5-HTTLPR is a limited component of the serotonergic system and normal transcription at this junction does not necessarily indicate that serotonin transmission is unimpaired at other points in the system. Future studies would need to consider the serotonin system more holistically in order to explain such unexpected findings.

Sampling differences across studies. Race and genetic heritage affect allelic distribution for 5-HTTLPR (Esau et al., 2008), while socio-economic status can be a key factor in the diagnostic procedure for ASD (Bertrand et al., 2001; Fombonne, 2003; Malcolm-Smith et al., 2013). It is therefore essential that demographic information is reported. Unfortunately, Tordjman et al. (2001) did not report demographic information for their sample beyond that they were all French Caucasians. Brune et al.'s (2006) study was also predominantly Caucasian, with only 13 of the 73 participants being from other races. The current sample showed greater racial diversity than either previous study, which is one of the advantages of conducting research in South Africa. Neither previous study reported socio-economic data, but it is noted that the current sample included children from a range of socio-economic backgrounds.

Our sample's allelic distribution was similar to that of a previous local ASD sample (Arieff et al., 2010). Our sample showed a higher incidence of the *s/s* genotype and of the short allele overall compared to Tordjman et al.'s (2001) sample. In contrast, the current protocol had a slightly lower incidence of the short allele than the Brune et al.'s (2006) study. Tordjman et al. (2001) reported that the short allele was related to greater impairment in

social communication and interaction. If this is the case, then the different rates of the short alleles across samples could indicate that the different studies unknowingly recruited different ASD subgroups (i.e. children with a specific phenotype in each study, but not the same phenotype across studies), which limits the comparability of findings. However, as was discussed above, the validity of the Tordjman et al. (2001) finding is questionable.

The current protocol purposefully recruited children with a range of verbal abilities and from different DSM-IV-TR ASD subtypes in an attempt to reflect the range of ASD. The inclusion of non-verbal children is considered an advantage of the current protocol as it increased the diversity of the sample. As both previous studies did not clearly outline the diversity of their samples, it is possible that they may have unknowingly recruited participants who represented a limited aspect of the spectrum and were not representative of the true diversity found in ASD.

The current protocol, and both previous studies on the role of 5-HTTLPR in ASD presentations had small group sizes of between 69 and 73 children. These sample sizes are too small to adequately represent the true 5-HTTLPR allelic distribution of the ASD population in each of the respective countries. For the current protocol we were able to compare our distribution to that of a local study that had a much larger sample size. These were compared and discussed previously, where it was noted that the current sample included less *l/l* genotypes and more *l/s* genotypes than the previous ASD study, but that our distribution was more in keeping with that of the local ASD population than the general South African population. Significantly, the current sample had over 20.00% higher rate of the *s/s* genotype than the general population.

Within in this protocol, no non-verbal children presented with the *s/s* genotype. As previous studies implicated the short allele in deficits in social communication, we had anticipated that the group with the most reduced serotonergic transmission (i.e. the *s/s* genotype) would include most non-verbal participants. The non-verbal group was very small, however, and this finding may well be due to sampling error.

As discussed above, race can be used as an indicator of different genetic heritage across participants, and different races have different 5-HTTLPR distributions. When samples are racially diverse, such as that of the current protocol, it is more difficult to obtain a sample that will be representative of the ASD population within that country. For instance, the current sample would ideally have had the same racial breakdown as the entire local ASD population, with sufficient participants in each racial group that the allelic distribution across that race in the country's ASD population was represented. This would require a large sample size for

each racial group, and an overall sample size that would be extremely difficult to obtain. Further, as local data on the prevalence of ASD is lacking, this approach is not possible at present.

Sampling biases are a limitation that future studies must always consider to ensure the generalisability of their findings. When these biases cannot be overcome, such as the case in ensuring an adequate sample size to fairly represent the racial diversity in South Africa and their different allelic frequencies, the bias must still be recognised.

The inclusion of non-verbal children in ASD research. This protocol used a design that allowed non-verbal children to be included in preliminary analyses and Study One. The separation of verbal and non-verbal participants is somewhat arbitrary as non-verbal participants should show the same type of impairment as the verbal participants, but to a greater degree for the social communication and interaction symptom domain of the DSM-5. Excluding these children therefore biases a sample as it does not reflect an entire segment of the dimension of severity in a core ASD symptom domain, and possibly a specific "non-verbal phenotype".

Although this study only recruited a small sample of non-verbal children, their place in this research must not be underestimated. Descriptively, non-verbal children showed greater deficits compared to the verbal participants in social communication and interaction, and for restricted and repetitive behaviours and interests. Considering the range of possible answers for each measure, both non-verbal and verbal participants showed the same scoring patterns: highest scores on the ASSP, followed by scores for the SCQ-Lifetime, and lowest scores for the RBS-R. It was not surprising then that when differences across genotypes within the non-verbal groups were qualitatively assessed 5-HTTLPR did not show an effect.

According to this data, non-verbal children are qualitatively similar to the verbal children with ASD as they show the same scoring patterns across measures – that is, they do not present with different symptom profiles other than that they are non-verbal. However, as they score as having higher deficits than the verbal children on all measures, this suggests that they place further along the dimension of deficits than verbal children and therefore represent a significant aspect of this disorder. The underrepresentation of non-verbal children in research indicates that studies are not recruiting participants who reflect the true range and diverse nature of this disorder.

The difficulties of including non-verbal children are understood as these limit the measures that can be used. However, researchers must endeavour to overcome these challenges and include such children in their research.

Study One conclusion. Study One did not find a relationship between 5-HTTLPR and overall severity in either of the DSM-5 core symptom domains of social communication and interaction or restricted and repetitive behaviours and interests.

The sample showed the expected increased incidence of the short allele, but no differences in core ASD symptoms across 5-HTTLPR genotypes or alleles were found. This study found that non-verbal participants exhibited the same pattern of impairment seen in the verbal children, but to a somewhat greater degree in both symptom domains.

Study One therefore concludes that reported findings that 5-HTTLPR relates to ASD symptom presentations were not replicated. The current protocol did not find relationships between 5-HTTLPR genotypes and/or alleles and impairment in social communication and interaction or in restricted and repetitive behaviours and interests in ASD.

The preliminary analyses highlighted the difficulties establishing phenotypes within ASD research, and discussed the specific difficulties experienced in this protocol in assessing impairment in core ASD symptoms. The broad approach used to assess core ASD symptoms here did not allow detailed phenotypes to be assessed, and genotype-phenotype relationships could therefore not be comprehensively assessed. Study One could only conclude that core ASD symptoms, as assessed broadly, were not related to 5-HTTLPR, but it cannot conclude that phenotypes do not exist for 5-HTTLPR genotypes.

Study Two: The Possible Roles of 5-HTTLPR in Core ASD symptoms and Theory of Mind

Study Two explored possible relationships between ToM and the core ASD symptoms described in the DSM-5, and between ToM and 5-HTTLPR. Studies on typically developing children have shown a positive correlation between ToM ability and social competence (Bosacki & Wilde Astington, 2001; Repacholi & Slaughter, 2003).

ToM is an ability that develops in a hierarchical way along a dimension. Early ability is indicated by joint attention, while more advanced ToM ability includes understanding social faux pas (Baron-Cohen et al., 1999; Frith & Frith, 2003). Much research, however, tends to treat ToM as a binary phenomenon: it is either present or absent. This is indicated by whether or not an individual is able to pass false-belief tasks (Baron-Cohen et al., 1985; Fombonne et al., 1994; Frith et al., 1994; Hoddenbach et al., 2012). Although the False-belief task remains a key test of mental state inference, this is an over-simplification of ToM. The UCT Autism Research Group developed a comprehensive ToM battery to measure ToM development more comprehensively (Hoogenhout & Malcolm-Smith, 2014). This battery

ensured that subtle differences in ToM ability were measured between participants, as it includes both early developing abilities (for example, pretend play) and skills that come online later in development (for example, the ability to differentiate lies and jokes).

Theory of Mind and core ASD symptoms. Study Two found no relationship between ToM and core ASD symptoms. Previous research on the relationship between ToM and social competence in ASD is mixed, with some finding a positive correlation (Fombonne et al., 1994; Frith et al., 1994) and others finding no relationship (Joseph & Tager-Flusberg, 2005). Studies which found relationships did not assess ASD symptom dimensions as per DSM-5, or DSM-IV-TR if the study was conducted prior to 2013, diagnostic criteria. Instead, the studies assessed specific behaviours, skills, or ASD characteristics. This study conducted a broader assessment of DSM-5 core symptoms, such that each individual was rated on their overall impairment for social communication and interaction, and for restricted and repetitive behaviours and interests. Despite ToM being comprehensively assessed, the measures used to try establish ASD phenotypes were problematic (discussed above). It is therefore possible that more subtle differences in symptom presentations, and how these relate to ToM, were not adequately measured in this protocol.

Theory of Mind and 5-HTTLPR. Study Two also assessed a possible role for 5-HTTLPR in ToM ability within ASD. It found no relationships between genotypes or alleles and ToM. It was expected that ToM would be most developed in the group with normal serotonergic transmission (i.e. the *l/l* genotype).

Age was considered as a possible mediating factor. ToM develops across the lifespan, and age needs to be considered in all ToM research. Within this sample age accounted for approximately 20.00% of the variability in ToM scores, suggesting that other factors must be involved. However, ToM development patterns did not differ across genotypes. Neither genotype nor allele strengthened regression models.

Unfortunately, IQ assessment was not included in this protocol. The literature has shown a relationship between IQ, and especially verbal IQ, and ToM in ASD (Fombonne et al., 1994; Happé, 1995). Further, a relationship exists between age and IQ in ASD. Assessment of ToM ability should ideally include consideration of age and IQ as mediators. It is possible that for this study IQ could explain a portion of the ToM variance currently attributed to age, and well as additional variance in ToM.

Study Two conclusion. Research into the possible relationships between 5-HTTLPR and ToM ability within ASD is at its absolute primacy: to my knowledge, no previous studies have explored these possible relationships. The current protocol therefore stands alone in

investigating possible relationships. As discussed in the Literature Review, serotonin is implicated in social functioning generally, and is implicated in ASD specifically. Further, ToM, a form of social cognition, is also implicated in ASD. It is therefore possible that 5-HTTLPR, as a mediator of serotonin, and ToM are related in ASD as both are implicated in social functioning. As deficits in social ability can be incapacitating in ASD, it is essential that research into possible mechanisms underlying these deficits are researched.

Although this study is limited by a small sample size, the ToM assessment was comprehensive. Age was a significant contributor to ToM ability. However, regression analyses did not indicate a role for 5-HTTLPR in ToM ability in this ASD sample.

Summary of All Findings

This protocol assessed the possible relationships between social communication and interaction, restricted and repetitive behaviours and interests, ToM, and 5-HTTLPR. No relationship between core symptoms and 5-HTTLPR emerged, or between ToM and 5-HTTLPR.

The absence of findings in this protocol was most likely due to the bluntness of the instruments used to measure core ASD symptoms, and problems utilizing these scales in the local context. The current protocol found that broadly assessing core ASD symptoms did not provide sufficient data to reveal relationships between ASD symptoms and genetic mechanisms. Possible relationships should not be dismissed until ASD characteristics are more comprehensively and finely measured. This will be attempted in later, larger studies into possible genotype-phenotype relationships between ASD characteristics and other candidate genes.

This protocol indicates, at most, that it is only the over-arching symptom domains that are not mediated by 5-HTTLPR. As discussed previously, these domains are so broad that it seems they are not useful in capturing subtle differences needed to identify and distinguish ASD phenotypes. A more refined approach is needed, and as current scales are too blunt to provide the necessary symptom data, new means to measure ASD characteristics will need to be developed.

To date explorations into ToM and different aspects of social competence in ASD have remained mixed. A relationship between social communication and ToM did not emerge in this protocol. Although ToM was comprehensively assessed, this needs to be done alongside measurement of ASD characteristics of equal specificity and sensitivity.

Finally, this protocol found that once age was considered, 5-HTTLPR did not contribute to ToM development in this sample.

5-HTTLPR and ToM are both implicated in ASD, but their roles remain unclear. ASD is a complex disorder and its underlying mechanisms remain elusive. The failure to conclusively characterise the role of ToM and 5-HTTLPR in this sample should not discourage further research in this area. Serotonin is clearly implicated in ASD and this protocol merely focused on the promoter region, a single and limited aspect of the serotonergic system. Numerous genes are implicated in ASD but their roles cannot be explained until research of this kind, which assesses for the specific aspects of ASD affected by each gene, is conducted.

The treatment and diagnostic procedures for ASD will continue to be limited until researchers are able to explain the fundamental, underlying structure and cause(s) of this disorder.

Limitations and Future Directions

Measures Used

The greatest limitation of this protocol was that the measures used to assess core ASD symptoms and characteristics were not sufficiently sensitive. We were only able to assess general performance within a diagnostic category, and could not distinguish subgroups or phenotypes - that is, our scales did not enable us to separate out phenotypes with different symptom profiles from one another. The current conceptualisation of ASD includes a very broad range of ASD characteristics in each core symptom domain. It is possible that the range of relevant characteristics within each core domain is too broad, and assessing impairment in these two domains will not reveal data that is specific enough to reveal phenotypes.

Future research should endeavour to find an approach that identifies key ASD characteristics necessary to establish phenotypes. This data should then be supplemented with further data on functioning ability (for example, IQ scores, verbal ability, activities of daily living) and on specifiers listed in the DSM-5 diagnostic criteria (American Psychiatric Association, 2013). Together this may allow better identification of different phenotypes, more specific relationships to be assessed, and these results may allow for far more targeted interventions.

As discussed above, it is also essential that parents understand the questions being asked if parent-reports are used. Low SES, literacy rates, and education regarding ASD will

all affect parents' level of understanding and hence their answers. Possible ways to overcome this would be to better educate parents on ASD or to assist parents in completing scales by conducting interviews. One could also move away from parent-report measures and instead use clinical observations, although this would affect the time and cost of the research.

DNA Collection and Genotyping

DNA was collected with cheek buccal swabs. As many children with ASD do not like to be touched and are wary of strangers, collecting these samples proved to be challenging. Despite efforts made to calm the children and allow them to overcome their fears, three children simply did not tolerate the swabs. In addition, many of the samples were undermined by a low DNA yield due to the non-verbal children having low salivation, and many of the other samples failed to genotype due to high bacteria counts as a result of poor oral hygiene (Hansen, Simonsen, Nielsen, & Hundrup, 2007).

It is suggested that researchers develop working relationships with doctors and hospitals caring for children with ASD and try gain access to blood drawn during routine check-ups. Children who did not tolerate cheek swabs are likely to be less cooperative with blood being drawn, so limited sample collection will still be expected. Blood samples, however, should yield higher DNA (Hansen et al., 2007). The blood-draw system therefore poses similar collection problems as the cheek swab system, but with fewer limitations for the processing of the DNA.

The use of genotyping without the inclusion of single-nucleotide polymorphism (SNP) analysis is a limitation in this protocol (Devlin et al., 2005; Heil & Schaaf, 2013; McCauley et al., 2004). This protocol established genotypes by measuring allele length. However, two alleles of the same length may contain different DNA sequences. SNP analysis is able to detect these differences. Genotyping without SNP analysis may artificially group individuals. Future studies should expand the genetic aspect of this protocol to include such differences and investigate the role they play in symptom presentations.

Further, 5-HTTLPR is only a single component in the serotonin system. Many other factors could influence the availability of serotonin in the central nervous system, such as the level of serotonin being produced, tryptophan depletion or loading through diet, the effect of medications, epigenetics, and several other genetic and hormonal influences. The full serotonin system needs to be explored in order for the role of serotonin in ASD to emerge.

Sample Used

This protocol faced limitations due to sampling bias. These biases were discussed in the preliminary analyses in regards to demographic data. Participants were of necessity restricted to those who were fluent in English or Afrikaans, and those within the Western Cape. Future studies should attempt to be more representative by assessing children in African languages, and by assessing in several areas across the country. As discussed previously, South Africa has a very diverse population, which makes it very difficult to recruit samples that reflect the national population. The constraints of a Masters study do not allow for such a sample to be recruited.

Limiting the protocol to the Western Cape may prevent results from being generalised to the rest of South Africa due to the cultural and racial diversity across our country. This also limited the recruitment pool and final sample size for the study. Ideally similar studies should be conducted in other provinces, both in rural and urban environments, with an emphasis on trying to recruit larger samples.

The sample size of the current protocol, as well as those of previous studies attempting to establish genotype-phenotype relationships between 5-HTTLPR and ASD have been relatively small, with Brune et al.'s (2006) study having the largest sample at 73 participants. In order to ensure future studies have sufficient power to identify even small effect sizes, sample sizes need to be much larger.

Due to the time constraints of a Masters thesis, long-term follow-up could not be conducted. This meant one could not establish if participants' symptom profiles remained consistent, and therefore if the role of 5-HTTLPR remains consistent across time and development. Ideally longitudinal studies would be included to allow follow up assessments to be conducted across development and into adulthood.

Summary and Conclusion

The current protocol investigated possible relationships between core ASD symptoms (social communication and interaction, and restricted and repetitive behaviours and interests), ToM, and 5-HTTLPR genotypes and alleles. This protocol is the first study within a larger study on the biological basis of social deficits in ASD. It thus took the logical first step of assessing ASD presentations in terms of the core symptom domains defined by DSM. One of the main aims of this study was to assess the usefulness of core ASD symptom scales in a

local sample, and several limitations were noted. Thereafter, statistical analyses did not reveal any significant effects or relationships for ASD core symptoms, 5-HTTLPR, and ToM.

It seems that approaching phenotyping using core symptom domains is unlikely to be useful. These domains are broad and include a range of symptoms of varying severity. Moving forward, it will be necessary to identify key characteristics within these domains and focus on assessing variability within those characteristics.

The scales used in the current protocol may provide some useful items regarding such key characteristics, but the appropriateness of such items will need to be assessed for the local parent population. Using direct observation of children's behaviour, as well as parent interviews would also be recommended.

The underlying causes of ASD remain elusive. Ultimately studies such as this one will continue with larger sample sizes and will expand to include other possible causes of ASD, such as other candidate genes and other psychological theories that have been posited. Each study of this nature will provide a step closer to understanding ASD, and this in turn will translate into improved diagnostics and interventions. As one of the most prevalent developmental disorders globally, the importance of progress in this area cannot be ignored.

References

Ackerman, B. P. (1981). Young children's understanding of a speaker's intentional use of a false utterance. *Developmental Psychology*, *17*(4), 472–480. doi:10.1037/0012-1649.17.4.472

Adamsen, D., Meili, D., Blau, N., Thöny, B., & Ramaekers, V. (2011). Autism associated with low 5-hydroxyindolacetic acid in CSF and the heterozygous SLC6A4 gene Gly56Ala plus 5-HTTLPR L/L promoter variants. *Molecular Genetics and Metabolism*, *102*(3), 368–73. doi:10.1016/j.ymgme.2010.11.162

American Psychiatric Association. (2000). *Diagnostics and statistical manual of mental disorders* (4th ed.). Washington, DC: Author.

American Psychiatric Association. (2013). *Diagnostics and statistical manual of mental disorders*. (Author, Ed.) (5th ed.). Washington, DC: Author.

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., & Zoghbi, H. Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature Genetics*, *23*(2), 185–8. doi:10.1038/13810

Arbelle, S. (2003). Relation of shyness in grade school children to the genotype for the long form of the serotonin transporter promoter region polymorphism. *American Journal of Psychiatry*, *160*(4), 671–676. doi:10.1176/appi.ajp.160.4.671

Arieff, Z., Kaur, M., Gameeldien, H., van der Merwe, L., & Bajic, V. B. (2010). 5-HTTLPR polymorphism: Analysis in South African autistic individuals. *Human Biology*, *82*(3), 291–300. doi:10.3378/027.082.0303

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Laucas, T., Meldrum, D., & Charman, T. (2006). Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: The Special Needs and Autism Project (SNAP). *The Lancet*, *368*, 210–215.

Baron-Cohen, S., Leslie, A. M., & Frith, U. (1985). Does the autistic child have a "theory of mind"? *Cognition*, *21*(1), 37–46.

Baron-Cohen, S., O'Riordan, M., Stone, V., Jones, R., & Plaisted, K. (1999). Recognition of faux pas by normally developing children and children with Asperger syndrome or high-functioning autism. *Journal of Autism and Developmental Disorders*, *29*(5), 407–18.

Bellini, S., & Hopf, A. (2007). The Development of the Autism Social Skills Profile: A preliminary analysis of psychometric properties. *Focus on Autism and Other Developmental Disabilities*, *22*(2), 80–87. doi:10.1177/10883576070220020801

Bertrand, J., Mars, A., Boyle, C., Bove, F., Yeargin-Allsopp, M., & Decoufle, P. (2001). Prevalence of autism in US populations: The brick township, New Jersey, investigation. *Pediatrics*, *108*(5), 1155–1161.

Berument, S. K., Rutter, M., Lord, C., Pickles, A., & Bailey, A. (1999). Autism screening questionnaire: Diagnostic validity. *The British Journal of Psychiatry*, *175*(5), 444–451. doi:10.1192/bjp.175.5.444

Bibby, H., & McDonald, S. (2005). Theory of mind after traumatic brain injury. *Neuropsychologia*, *43*(1), 99–114. doi:10.1016/j.neuropsychologia.2004.04.027

Bolton, P. F., Carcani-Rathwell, I., Hutton, J., Goode, S., Howlin, P., & Rutter, M. (2011). Epilepsy in autism: Features and correlates. *The British Journal of Psychiatry*, *198*(4), 289–94. doi:10.1192/bjp.bp.109.076877

Boomsma, A., Van Lang, N. D. J., De Jonge, M. V, De Bildt, a a, Van Engeland, H., & Minderaa, R. B. (2008). A new symptom model for autism cross-validated in an independent sample. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *49*(8), 809–816. doi:10.1111/j.1469-7610.2008.01897.x

Bosacki, S., & Wilde Astington, J. (2001). Theory of Mind in preadolescence: Relations between social understanding and social competence. *Social Development*, *8*(2), 237–255. doi:10.1111/1467-9507.00093

Boyle, C. A., Boulet, S., Schieve, L. A., Cohen, R. A., Blumberg, S. J., Yeargin-Allsopp, M., … Kogan, M. D. (2011). Trends in the prevalence of developmental disabilities in US Children, 1997–2008. *Pediatrics*, *127*(6), 1034–1042. doi:10.1542/peds.2010-2989

Bozalek, F., & Malcolm-Smith, S. (2014). *Autism Screening in Children: Using the Social Communication Questionnaire in a Western Cape Population*. University of Cape Town.

Brock, M. E., Freuler, A., Baranek, G. T., Watson, L. R., Poe, M. D., & Sabatino, A. (2012). Temperament and sensory features of children with autism. *Journal of Autism and Developmental Disorders*, *42*(11), 2271–84. doi:10.1007/s10803-012-1472-5

Brooks, B. L., Sherman, E. M. S., & Strauss, E. (2009). NEPSY-II: A Developmental Neuropsychological Assessment, Second Edition. *Child Neuropsychology*, *16*(1), 80–101. doi:10.1080/09297040903146966

Brune, C. W., Kim, S.-J., Salt, J., Leventhal, B. L., Lord, C., & Cook, E. H. (2006). 5-HTTLPR genotype-specific phenotype in children and adolescents with autism. *The American Journal of Psychiatry*, *163*(12), 2148–2156. doi:10.1176/appi.ajp.163.12.2148

Brüne, M., & Brüne-Cohrs, U. (2006). Theory of mind - evolution, ontogeny, brain mechanisms and psychopathology. *Neuroscience and Biobehavioral Reviews*, *30*(4), 437–455. doi:10.1016/j.neubiorev.2005.08.001

Burgess, N. K., Sweeten, T. L., McMahon, W. M., & Fujinami, R. S. (2006). Hyperserotoninemia and altered immunity in autism. *Journal of Autism and Developmental Disorders*, *36*(5), 697–704. doi:10.1007/s10803-006-0100-7

Canli, T., & Lesch, K.-P. (2007). Long story short: The serotonin transporter in emotion regulation and social cognition. *Nature Neuroscience*, *10*(9), 1103–9. doi:10.1038/nn1964

Centers for Disease Control and Prevention. (2012). Prevalence of autism spectrum disorders - Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008. *Morbidity and Mortality Weekly Report*, *61*(3), 1–19.

Cermak, S. A., Curtin, C., & Bandini, L. G. (2013). Food selectivity and sensory sensitivity in children with autism spectrum disorders. *Journal of American Dietetic Association*, *110*(2), 238–246. doi:10.1016/j.jada.2009.10.032.Food

Chakrabarti, S., & Fombonne, E. (2005). Pervasive developmental disorders in preschool children: confirmation of high prevalence. *The American Journal of Psychiatry*, *162*(6), 1133–41. doi:10.1176/appi.ajp.162.6.1133

Chandler, S., Carcani-Rathwell, I., Charman, T., Pickles, A., Loucas, T., Meldrum, D., … Baird, G. (2013). Parent-reported gastro-intestinal symptoms in children with autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *43*(12), 2737–47. doi:10.1007/s10803-013-1768-0

Chandler, S., Charman, T., Baird, G., Simonoff, E., Loucas, T., Meldrum, D., … Pickles, A. (2007). Validation of the Social Communication Questionnaire in a population cohort of children with autism spectrum disorders. *American Academy of Child and Adolescent Psychiatry*, *46*, 1324–1332.

Charman, T., Baron-Cohen, S., Swettenham, J., Baird, G., Cox, A., & Drew, A. (2000). Testing joint attention, imitation, and play as infancy precursors to language and theory of mind. *Cognitive Development*, *15*(4), 481–498. doi:10.1016/S0885-2014(01)00037-5

Cho, S.-C., Yoo, H. J., Park, M., Cho, I. H., Kim, B.-N., Kim, J.-W., … Kim, S. A. (2011). Genome-wide association scan of korean autism spectrum disorders with language delay: a preliminary study. *Psychiatry Investigation*, *8*(1), 61–6. doi:10.4306/pi.2011.8.1.61

Cook, E. H., & Leventhal, B. L. (1996). The serotonin system in autism. *Current Opinions in Pediatrics*, *8*, 348–354.

Cross-Disorder Group of the Psychiatric Genomics Consortium. (2013). Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet*, *381*(9875), 1371–1379. doi:10.1016/S0140-6736(12)62129-1

Devlin, B., Cook, E. H., Coon, H., Dawson, G., Grigorenko, E. L., McMahon, W., … Schellenberg, G. D. (2005). Autism and the serotonin transporter: the long and short of it. *Molecular Psychiatry*, *10*(12), 1110–6. doi:10.1038/sj.mp.4001724

Devries, R. (1970). The development of role-taking as reflected by behaviour of bright, average, and retarded children in a social guessing game. *Child Development*, *41*(3), 759–770. doi:10.1111/1467-8624

Eaves, L. C., Wingert, H. D., Ho, H. H., & Mickelson, E. C. R. (2006). Screening for autism spectrum disorders with the social communication questionnaire. *Journal of Developmental and Behavioral Pediatrics : JDBP*, *27*(2), S95–S103.

Edwards, D. H., & Kravitz, E. A. (1997). Serotonin , social status and aggresion. *Current Opinion in Neurobiology*, *7*, 812–819.

Eisenmajer, R., Prior, M., Leekam, S. R., Wing, L., Gould, J., Weaver, L. K., & Ong, B. (1996). Comparison of clinical symptoms in autism and Asperger's disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, *35*(11), 1523–1531.

Esau, L., Kaur, M., Adonis, L., & Arieff, Z. (2008). The 5-HTTLPR polymorphism in South African healthy populations: A global comparison. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *115*(5), 755–60. doi:10.1007/s00702-007-0012-5

Field, A. (2009). *Discovering Statistics Using SPSS*. London, England: SAGE.

Fombonne, E. (2003). Epidemiological surveys of autism and other pervasive developmental disorders: An update. *Journal of Autism and Developmental Disorders*, *33*(4), 365–82.

Fombonne, E., Siddons, F., Achard, S., Frith, U., & Happé, F. (1994). Adaptive behaviour and theory of mind in autism. *European Child and Adolescent Psychiatry*, *3*(3), 176–186.

Frazier, T. W., Youngstrom, E. a, Kubu, C. S., Sinclair, L., & Rezai, A. (2008). Exploratory and confirmatory factor analysis of the autism diagnostic interview-revised. *Journal of Autism and Developmental Disorders*, *38*(3), 474–80. doi:10.1007/s10803-007-0415-z

Frazier, T. W., Youngstrom, E. a, Speer, L., Embacher, R., Law, P., Constantino, J., … Eng, C. (2012). Validation of proposed DSM-5 criteria for autism spectrum disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, *51*(1), 28–40.e3. doi:10.1016/j.jaac.2011.09.021

Frith, U., & Frith, C. D. (2003). Development and neurophysiology of mentalizing. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *358*(1431), 459–473. doi:10.1098/rstb.2002.1218

Frith, U., Happé, F., & Siddons, F. (1994). Autism and theory of mind in everyday life. *Social Development*, *3*(2), 108–124. doi:10.1111/j.1467-9507.1994.tb00031.x

Fung, L. K., Chahal, L., Libove, R. a, Bivas, R., & Hardan, A. Y. (2012). A retrospective review of the effectiveness of aripiprazole in the treatment of sensory abnormalities in autism. *Journal of Child and Adolescent Psychopharmacology*, *22*(3), 245–248. doi:10.1089/cap.2010.0103

Gabriels, R. L., Cuccaro, M. L., Hill, D. E., Ivers, B. J., & Goldson, E. (2005). Repetitive behaviors in autism: relationships with associated clinical features. *Research in Developmental Disabilities*, *26*(2), 169–81. doi:10.1016/j.ridd.2004.05.003

Gau, S. S.-F., Lee, C.-M., Lai, M.-C., Chiu, Y.-N., Huang, Y.-F., Kao, J.-D., & Wu, Y.-Y. (2011). Psychometric properties of the Chinese version of the Social Communication Questionnaire. *Research in Autism Spectrum Disorders*, *5*(2), 809–818. doi:10.1016/j.rasd.2010.09.010

Geschwind, D. H. (2009). Advances in autism. *Annual Review of Medicine*, *60*, 367–380. doi:10.1146/annurev.med.60.053107.121225

Ghaziuddin, M. (2010). Should the DSM V drop Asperger syndrome? *Journal of Autism and Developmental Disorders*, *40*(9), 1146–8. doi:10.1007/s10803-010-0969-z

Greenberg, B. D., Li, Q., Lucas, F. R., Hu, S., Sirota, L. a, Benjamin, J., … Murphy, D. L. (2000). Association between the serotonin transporter promoter polymorphism and personality traits in a primarily female population sample. *American Journal of Medical Genetics*, *96*(2), 202–16.

Hanley, H. G., Stahl, S. M., & Freedman, D. X. (1977). Hyperserotonemia and amine metabolites in autistic and retarded children. *Archives of General Psychiatry*, *34*(5), 521–531. doi:10.1001/archpsyc.1977.01770170031002

Hansen, T. V. O., Simonsen, M. K., Nielsen, F. C., & Hundrup, Y. A. (2007). Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. *Cancer Epidemiology, Biomarkers & Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, *16*(10), 2072–6. doi:10.1158/1055-9965.EPI-07-0611

Happé, F. (1993). Communicative competence and theory of mind in autism: A test of relevance theory. *Cognition*, *48*(2), 101–119.

Happé, F. (1994). An advanced test of theory of mind: Understanding of story characters' thoughts and feelings by able autistic, mentally handicapped, and normal children and adults. *Journal of Autism and Developmental Disorders*, *24*(2), 129–54.

Happé, F. (1995). The role of age and verbal ability in the theory of mind task performance of subjects with autism. *Child Development*, *66*(3), 843–55.

Happé, F., & Frith, U. (1996). The neuropsychology of autism. *Brain*, *119*(4), 1377–1400.

Happé, F., & Frith, U. (2006). The weak coherence account: Detail-focused cognitive style in autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *36*, 5–25.

Heil, K. M., & Schaaf, C. P. (2013). The genetics of Autism Spectrum Disorders - a guide for clinicians. *Current Psychiatry Reports*, *15*(1), 334. doi:10.1007/s11920-012-0334-3

Higley, J. D., King, S. T., Hasert, M. F., Champoux, M., Suomi, S. J., & Linnoila, M. (1996). Stability of interindividual differences in serotonin function and its relationship to severe sggression and competent social. *Neuropsychopharmacology*, *14*(1), 67–76.

Hill, P. M., & McCune-Nicolich, L. (1981). Pretend play and patterns of cognition in Down's syndrome children. *Child Development*, *52*(2), 611–617. doi:10.2307/1129181

Hoddenbach, E., Koot, H. M., Clifford, P., Gevers, C., Clauser, C., Boer, F., & Begeer, S. (2012). Individual differences in the efficacy of a short theory of mind intervention for children with autism spectrum disorder: A randomized controlled trial. *Trials*, *13*, 206–219. doi:10.1186/1745-6215-13-206

Hoogenhout, M., & Malcolm-Smith, S. (2014). Theory of mind in autism spectrum disorder: Does DSM classification predict development? *Research in Autism Spectrum Disorders*, *8*(6), 597–607. doi:10.1016/j.rasd.2014.02.005

Howlin, P. (2003). Outcome in high-functioning adults with autism with and without early language delays: implications for the differentiation between autism and Asperger syndrome. *Journal of Autism and Developmental Disorders*, *33*(1), 3–13.

Huang, C. H., & Santangelo, S. L. (2008). Autism and serotonin transporter gene polymorphisms: a systematic review and meta-analysis. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics : The Official Publication of the International Society of Psychiatric Genetics*, *147B*(6), 903–13. doi:10.1002/ajmg.b.30720

Huerta, M., Bishop, S. L., Duncan, A., Hus, V., & Lord, C. (2012). Application of DSM-5 criteria for autism spectrum disorder to three samples of children with DSM-IV

diagnoses of pervasive developmental disorders. *American Journal of Psychiatry*, *169*(10), 1056–64. doi:10.1176/appi.ajp.2012.12020276

Hutchins, T. L., Prelock, P. a, & Bonazinga, L. (2012). Psychometric evaluation of the Theory of Mind Inventory (ToMI): A study of typically developing children and children with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *42*(3), 327–41. doi:10.1007/s10803-011-1244-7

Jacob, C. P. (2004). Association between allelic variation of serotonin transporter function and neuroticism in anxious Cluster C Personality Disorders. *American Journal of Psychiatry*, *161*(3), 569–572. doi:10.1176/appi.ajp.161.3.569

Joseph, R. M., & Tager-Flusberg, H. (2005). The relationship of theory of mind and executive functions to symptom type and severity in children with autism. *Developmental Psychopathology*, *16*(1), 137–155.

Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child*, *2*(3), 217–250.

Kendler, K. S. (2010). Advances in our understanding of genetic risk factors for autism spectrum disorders. *American Journal of Psychiatry*, *167*(11), 1291–1293. doi:10.1176/appi.ajp.2010.10081160

Kim, D. K., Lim, S. W., Lee, S., Sohn, S. E., Kim, S., Hahn, C. G., & Carroll, B. J. (2000). Serotonin transporter gene polymorphism and antidepressant response. *NeuroReport*, *11*(1), 215–219.

Kim, Y. S., Leventhal, B. L., Koh, Y.-J., Fombonne, E., Laska, E., Lim, E.-C., … Grinker, R. R. (2011). Prevalence of Autism Spectrum Disorders in a total population sample. *American Journal of Psychiatry*, *168*(9), 904–912. doi:10.1176/appi.ajp.2011.10101532

Klauck, S., Poustka, F., Benner, A., Lesch, K., & Poustka, A. (1997). Serotonin transporter (5-HTT) gene variants associated with autism? *Human Molecular Genetics*, *6*(13), 2233–2238. doi:10.1093/hmg/6.13.2233

Klin, A., Volkmar, F. R., & Sparrow, S. S. (1992). Autistic social dysfunction: Some limitations of the Theory of Mind hypothesis. *Journal of Child Psychology and Psychiatry*, *33*(5), 861–876. doi:10.1111/j.1469-7610.1992.tb01961.x

Knivsberg, A. M., Reichelt, K. L., Hoien, T., & Nodland, M. (2002). A randomised, controlled study of dietary intervention in autistic syndromes. *Nutritional Neuroscience*, *5*(4), 251–261.

Kogan, M. D., Blumberg, S. J., Schieve, L. A., Boyle, C. A., Perrin, J. M., Ghandour, R. M., … van Dyck, P. C. (2009). Prevalence of parent-reported diagnosis of autism spectrum

disorder among children in the US, 2007. *Pediatrics*, *124*(5), 1395–403. doi:10.1542/peds.2009-1522

Kolevzon, A. (2006). Selective serotonin reuptake inhibitors in autism: A review of efficacy and tolerability. *The Journal of Clinical Psychiatry*, *67*(3), 407–414.

Krakowiak, P., Goodlin-Jones, B., Hertz-Picciotto, I., Croen, L. A., & Hansen, R. L. (2008). Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study. *Journal of Sleep Research*, *17*(2), 197–206. doi:10.1111/j.1365-2869.2008.00650.x

Kral, T. V. E., Eriksen, W. T., Souders, M. C., & Pinto-Martin, J. A. (2013). Eating behaviors, diet quality, and gastrointestinal symptoms in children with autism spectrum disorders: a brief review. *Journal of Pediatric Nursing*, *28*(6), 548–556. doi:10.1016/j.pedn.2013.01.008

Kruesi, M. J. P., Rapoport, J. L., Hamburger, S., Hibbs, E., Potter, W. Z., Lenane, M., & Brown, G. L. (1990). Cerebrospinal fluid monoamine metabolites, aggression, and impulsivity in disruptive behavior disorders of children and adolescents. *Archives of Disease in Childhood*, *47*(5), 419–426. doi:10.1001/archpsyc.1990.01810170019003

Lam, K. S. L. (2004). *The Repetitive Behavior Scale - Revised: Independent validation and the effects of subject variables*. The Ohio State University, Columbus.

Lam, K. S. L., & Aman, M. G. (2007). The Repetitive Behavior Scale-Revised: Independent validation in individuals with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *37*(5), 855–66. doi:10.1007/s10803-006-0213-z

Lerner, M. D., Hutchins, T. L., & Prelock, P. a. (2011). Brief report: preliminary evaluation of the theory of mind inventory and its relationship to measures of social skills. *Journal of Autism and Developmental Disorders*, *41*(4), 512–7. doi:10.1007/s10803-010-1066-z

Leslie, A. M. (1987). Pretense and representation: The origins of "theory of mind." *Psychological Review*, *94*(4), 412–426. doi:10.1037/0033-295X.94.4.412

Liss, M., Saulnier, C., Fein, D., & Kinsbourne, M. (2006). Sensory and attention abnormalities in autistic spectrum disorders. *Autism: The International Journal of Research and Practice*, *10*(2), 155–72. doi:10.1177/1362361306062021

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: A revision version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disordrs. *Journal of Autism and Developmental Disorders*, *24*(5), 659–685.

Macintosh, K., & Dissanayake, C. (2006). Social skills and problem behaviours in school aged children with high-functioning autism and Asperger's Disorder. *Journal of Autism and Developmental Disorders*, *36*(8), 1065–76. doi:10.1007/s10803-006-0139-5

Malcolm-Smith, S., Hoogenhout, M., Ing, N., Thomas, K. G., & de Vries, P. (2013). Autism spectrum disorders - Global challenges and local opportunities. *Journal of Child and Adolescent Mental Health*, *25*(1), 1–5. doi:10.2989/17280583.2013.767804

Mandy, W. P. L., Charman, T., & Skuse, D. H. (2012). Testing the construct validity of proposed criteria for DSM-5 autism spectrum disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, *51*(1), 41–50. doi:10.1016/j.jaac.2011.10.013

Manjiviona, J., & Prior, M. (1995). Comparison of Asperger syndrome and high-functioning autistic children on a test of motor impairment. *Journal of Autism and Developmental Disorders*, *25*(1), 23–39.

Mann, J. J. (1999). Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. *Neuropsychopharmacology*, *21*(2), 99S–105S. doi:10.1016/S0893-133X(99)00040-8

Matson, J. L., & Boisjoli, J. A. (2007). Differential diagnosis of PDDNOS in children. *Research in Autism Spectrum Disorders*, *1*(1), 75–84. doi:10.1016/j.rasd.2006.09.001

Matson, J. L., Hattier, M. a, & Williams, L. W. (2012). How does relaxing the algorithm for autism affect DSM-V prevalence rates? *Journal of Autism and Developmental Disorders*, *42*(8), 1549–56. doi:10.1007/s10803-012-1582-0

Mayes, S. D., Calhoun, S. L., & Crites, D. L. (2001). Does DSM-IV asperger's disorder exist? *Jounral of Abnormal Child Psychology*, *29*(3), 263–271.

McCauley, J. L., Olson, L. M., Dowd, M., Amin, T., Steele, a, Blakely, R. D., … Sutcliffe, J. S. (2004). Linkage and association analysis at the serotonin transporter (SLC6A4) locus in a rigid-compulsive subset of autism. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics : The Official Publication of the International Society of Psychiatric Genetics*, *127B*(1), 104–12. doi:10.1002/ajmg.b.20151

McDougle, C., Naylor, S. T., Cohen, D. J., Aghajanian, G. K., Heninger, G. R., & Price, L. H. (1996). Effects of tryptophan depletion in drug-free adults with autistic disorder. *Archives of General Psychiatry*, *53*(11), 993–1000. doi:10.1001/archpsyc.1996.01830110029004

Meyer, J. A., & Minshew, N. J. (2002). An update on neurocognitive profiles in Asperger Syndrome and High-Functioning Autism. *Focus on Autism and Other Developmental Disabilities*, *17*(3), 152–160. doi:10.1177/10883576020170030501

Miller, J. N., & Ozonoff, S. (2000). The external validity of Asperger disorder: Lack of evidence from the domain of neuropsychology. *Journal of Abnormal Psychology*, *109*(2), 227–238. doi:10.1037//0021-843X.109.2.227

Mirenda, P., Smith, I. M., Vaillancourt, T., Georgiades, S., Duku, E., Szatmari, P., … Zwaigenbaum, L. (2010). Validating the Repetitive Behavior Scale-revised in young children with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *40*(12), 1521–30. doi:10.1007/s10803-010-1012-0

Mody, M., Manoach, D. S., Guenther, F. H., Kenet, T., Bruno, K. a, McDougle, C. J., & Stigler, K. A. (2013). Speech and language in autism spectrum disorder: A view through the lens of behavior and brain imaging. *Neuropsychiatry*, *3*(2), 223–232. doi:10.2217/npy.13.19

Muhle, R., Trentacoste, S., & Rapin, I. (2004). The genetics of autism. *Pediatrics*, *113*, 472–486.

Myer, L., Ehrlich, R. I., & Susser, E. S. (2004). Social epidemiology in South Africa. *Epidemiologic Reviews*, *26*, 112–23. doi:10.1093/epirev/mxh004

Myer, L., Stein, D. J., Grimsrud, A., Seedate, S., & Williams, D. R. (2011). Social determinants of psychological distress in a nationally-representative sample of South African adults. *Social Science & Medicine*, *66*(8), 1828–1840. doi:10.1016/j.socscimed.2008.01.025.Social

Naito, M., Komatsu, S., & Fuke, T. (1994). Normal and autistic children's understanding of their own and others' false belief : A study from Japan. *British Journal of Developmental Psychology*, *12*, 403–416.

Noterdaeme, M., Wriedt, E., & Höhne, C. (2010). Asperger's syndrome and high-functioning autism: Language, motor and cognitive profiles. *European Child and Adolescent Psychiatry*, *19*(6), 475–81. doi:10.1007/s00787-009-0057-0

Osborn, A. G. (2012). *Osborn's Brain: Imaging, Pathology, and Anatomy*. Salt Lake City: Amirsys Publishing.

Ozonoff, S., & McEvoy, R. E. (1994). A longitudinal study of executive function and theory of mind development in autism. *Development and Psychopathology*, *6*(03), 415–431. doi:10.1017/S0954579400006027

Patkar, A. A., Berrettini, W. H., Hoehe, M., Thornton, C. C., Gottheil, E., Hill, K., & Weinstein, S. P. (2002). Serotonin transporter polymorphisms and measures of impulsivity, aggression, and sensation seeking among African-American cocaine-dependent individuals. *Psychiatry Research*, *110*(2), 103–15.

Peacock, G., Amendah, D., Ouyang, L., & Grosse, S. D. (2012). Autism spectrum disorders and health care expenditures: The effects of co-occurring conditions. *Journal of Developmental and Behavioral Pediatrics*, *33*(1), 2–8. doi:10.1097/DBP.0b013e31823969de

Pérez-Edgar, K., Bar-haim, Y., Mcdermott, J. M., Gorodetsky, E., Hodgkinson, A., Goldman, D., … Fox, N. A. (2011). Variations in the serotonin transporter gene are associated wtih attention bias patterns to positive and negative emotion faces. *Biological Psychology*, *83*(3), 269–271. doi:10.1016/j.biopsycho.2009.08.009.Variations

Perner, J., Leekam, S. R., & Wimmer, H. (1987). Three-year-olds' difficulty with false belief: The case for a conceptual deficit. *British Journal of Developmental Psychology*, *5*(2), 125–137. doi:10.1111/j.2044-835X.1987.tb01048.x

Poelmans, G., Franke, B., Pauls, D. L., Glennon, J. C., & Buitelaar, J. K. (2013). AKAPs integrate genetic findings for autism spectrum disorders. *Translational Psychiatry*, *3*(6), 270. doi:10.1038/tp.2013.48

Pollio, M. R., & Pollio, H. R. (2008). A test of metaphoric comprehension and some preliminary developmental data. *Journal of Child Language*, *6*(01), 111–120. doi:10.1017/S0305000900007650

Pratt, C., & Bryant, P. (1990). Young children understand that looking leads to knowing (so long as they are looking into a single barrel). *Child Development*, *61*, 973–982.

Prior, M., Leekam, S., Ong, B., Eisenmajer, R., Wing, L., Gould, J., & Dowe, D. (1998). Are there subgroups within the autistic spectrum? A cluster analysis of a group of children with autistic spectrum disorders. *Journal of Child Psychology and Psychiatry*, *39*(6), 893–902. doi:10.1111/1469-7610.00389

Rapin, I., & Dunn, M. (2003). Update on the language disorders of individuals on the autistic spectrum. *Brain and Development*, *25*(3), 166–172. doi:10.1016/S0387-7604(02)00191-2

Reneman, L., Schilt, T., de Win, M. M., Booij, J., Schmand, B., van den Brink, W., & Bakker, O. (2006). Memory function and serotonin transporter promoter gene polymorphism in ecstasy (MDMA) users. *Journal of Psychopharmacology*, *20*(3), 389–399. doi:10.1177/0269881106063266

Repacholi, B., & Slaughter, V. (2003). *Individual differences in theory of mind: Implications for typical and atypical development* (Vol. Psychology). Sussex.

Rice, C. (2009). *Prevalence of Autism Spectrum Disorders: Autism and Developmental Disabilities Monitoring Network, United States, 2006. Morbidity and Mortality Weekly Report. Surveillance Summaries. Volume 58, Number SS-10*. Centers for Disease Control and Prevention.

Richdale, A. L. (1999). Sleep problems in autism: Prevalence, cause, and intervention. *Developmental Medicine & Child Neurology*, *41*(1), 60–66. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10068053

Ropper, A. H., & Samuels, M. A. (2009). *Adams & Victor's Principles of Neurology*. USA: McGraw-Hill Prof Med/Tech.

Rutter, M., Bailey, A., & Lord, C. (2003). *Social Communication Questionnaire*. (Services Western Psychological, Ed.). Los Angeles.

Scheeren, A. M., de Rosnay, M., Koot, H. M., & Begeer, S. (2013). Rethinking theory of mind in high-functioning autism spectrum disorder. *Journal of Child Psychology and Psychiatry*, *54*(6), 628–635. doi:10.1111/jcpp.12007

Schreck, K. A., Mulick, J. A., & Smith, A. F. (2004). Sleep problems as possible predictors of intensified symptoms of autism. *Research in Developmental Disabilities*, *25*(1), 57–66. doi:10.1016/j.ridd.2003.04.007

Sen, S., Burmeister, M., & Ghosh, D. (2004). Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics : The Official Publication of the International Society of Psychiatric Genetics*, *127B*(1), 85–9. doi:10.1002/ajmg.b.20158

Senju, A. (2012). Spontaneous theory of mind and its absence in autism spectrum disorders. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *18*(2), 108–113. doi:10.1177/1073858410397208

Sharma, J. R., Arieff, Z., Gameeldien, H., Davids, M., Kaur, M., & van der Merwe, L. (2013). Association analysis of two single-nucleotide polymorphisms of the RELN gene with autism in the South African population. *Genetic Testing and Molecular Biomarkers*, *17*(2), 93–98. doi:10.1089/gtmb.2012.0212

Simonoff, E., Pickles, A., Charman, T., Chandler, S., Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with autism spectrum disorders: Prevalence, comorbidity, and associated factors in a population-derived sample. *Journal of the*

American Academy of Child and Adolescent Psychiatry, *47*(8), 921–9. doi:10.1097/CHI.0b013e318179964f

Snow, A. V, Lecavalier, L., & Houts, C. (2009). The structure of the Autism Diagnostic Interview-Revised: Diagnostic and phenotypic implications. *Journal of Child Psychology and Psychiatry*, *50*(6), 734–742. doi:10.1111/j.1469-7610.2008.02018.x

Springer, P. E., van Toorn, R., Laughton, B., & Kidd, M. (2013). Characteristics of children with pervasive developmental disorders attending a developmental clinic in the Western Cape Province, South Africa. *South African Journal of Child Health*, *7*(3), 95–99. doi:10.7196/SAJCH.530

SPSS Inc. (2013). *SPSS Statistics*. Chicago, IL: IBM.

Statistics South Africa. (2012). *South African National Census of 2011*. Pretoria: Statistics South Africa. Retrieved from http://www.statssa.gov.za/publications/P03014/P030142011.pdf

Steele, S., Joseph, R. M., & Tager-Flusberg, H. (2003). Brief report: Developmental change in theory of mind abilities in children with autism. *Journal of Autism and Developmental Disorders*, *33*(4), 461–467.

Stone, V. E., Baron-Cohen, S., & Knight, R. T. (1998). Frontal lobe contributions to theory of mind. *Journal of Cognitive Neuroscience*, *10*(5), 640–656.

Sugie, Y., Sugie, H., Fukuda, T., Ito, M., Sasada, Y., Nakabayashi, M., … Ohzeki, T. (2005). Clinical efficacy of Fluvoxamine and functional polymorphism in a serotonin transporter gene on childhood autism. *Journal of Autism and Developmental Disorders*, *35*(3), 377–385. doi:10.1007/s10803-005-3305-2

Swedo, S., Thorsen, P., & Pine, D. (2008). Autism and Other Pervasive Developmental Disorders Conference. In *American Psychiatric Institute for Research and Education [APIRE] conference series "The Future of Psychiatric Diagnosis: Refining the Research Agenda."* M.I.N.D. Institute, Sacramento, California.

Szatmari, P., Georgiades, S., Bryson, S., Zwaigenbaum, L., Roberts, W., Mahoney, W., … Tuff, L. (2006). Investigating the structure of the restricted, repetitive behaviours and interests domain of autism. *Journal of Child Psychology and Psychiatry*, *47*(6), 582–590. doi:10.1111/j.1469-7610.2005.01537.x

Talay-Ongan, A., & Wood, K. (2000). Unusual sensory sensitivities in autism: A possible crossroads. *International Journal of Disability, Development and Education*, *47*(2), 201–212.

Thede, L. L., & Coolidge, F. L. (2007). Psychological and neurobehavioral comparisons of children with Asperger's Disorder versus High-Functioning Autism. *Journal of Autism and Developmental Disorders*, *37*(5), 847–54. doi:10.1007/s10803-006-0212-0

Tordjman, S., Gutknecht, L., Carlier, M., Spitz, E., Antoine, C., Slama, F., … Anderson, G. M. (2001). Role of the serotonin transporter gene in the behavioral expression of autism. *Molecular Psychiatry*, *6*(4), 434–9. doi:10.1038/sj.mp.4000873

Van Lang, N. D. J., Boomsma, A., Sytema, S., de Bildt, A. a, Kraijer, D. W., Ketelaars, C., & Minderaa, R. B. (2006). Structural equation analysis of a hypothesised symptom model in the autism spectrum. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *47*(1), 37–44. doi:10.1111/j.1469-7610.2005.01434.x

Verhoeven, J. S., Rommel, N., Prodi, E., Leemans, A., Zink, I., Vandewalle, E., … Sunaert, S. (2012a). Is there a common neuroanatomical substrate of language deficit between autism spectrum disorder and specific language impairment? *Cerebral Cortex*, *22*(10), 2263–2271. doi:10.1093/cercor/bhr292

Verhoeven, J. S., Rommel, N., Prodi, E., Leemans, A., Zink, I., Vandewalle, E., … Sunaert, S. (2012b). Is there a common neuroanatomical substrate of language deficit between autism spectrum disorder and specific language impairment? *Cerebral Cortex*, *22*(10), 2263–2271. doi:10.1093/cercor/bhr292

Wellman, H. M., Cross, D., & Watson, J. (2001). Meta-analysis of theory of mind development: The truth about false belief. *Child Development*, *72*(3), 655–684.

Wellman, H. M., & Liu, D. (2004). Scaling of Theory-of-Mind tasks. *Child Development*, *75*(2), 523–541. doi:10.1111/j.1467-8624.2004.00691.x

Wellman, H. M., & Woolley, J. D. (1990). From simple desires to ordinary beliefs: The early development of everyday psychology. *Cognition*, *35*, 245–275.

White, J. F. (2003). Intestinal pathophysiology in autism. *Experimental Biology and Medicine*, *228*(6), 639–649.

Williams, J. G., Higgins, J. P. T., & Brayne, C. E. G. (2006). Systematic review of prevalence studies of autism spectrum disorders. *Archives of Disease in Childhood*, *91*(1), 8–15. doi:10.1136/adc.2004.062083

Williams, K., Tuck, M., Helmer, M., Bartak, L., Mellis, C., & Peat, J. K. (2008). Diagnostic labelling of autism spectrum disorders in NSW. *Journal of Paediatrics and Child Health*, *44*(3), 108–13. doi:10.1111/j.1440-1754.2007.01232.x

Wimmer, H., & Perner, J. (1983). Beliefs about beliefs: Representation and constraining function of wrong beliefs in young children's understanding of deception. *Cognition*, *13*(1), 103–128. doi:10.1016/0010-0277(83)90004-5

Wing, L., Gould, J., & Gillberg, C. (2013). Autism spectrum disorders in the DSM-V: better or worse than the DSM-IV? *Research in Developmental Disabilities*, *32*(2), 768–73. doi:10.1016/j.ridd.2010.11.003

Winner, E., Brownell, H., Happé, F., Blum, A., & Pincus, D. (1998). Distinguishing lies from jokes: Theory of mind deficits and discourse interpretation in right hemisphere brain-damaged patients. *Brain and Language*, *62*(1), 89–109. doi:10.1006/brln.1997.1889

Witwer, A. N., & Lecavalier, L. (2007). Autism screening tools: An evaluation of the Social Communication Questionnaire and the Developmental Behaviour Checklist-Autism Screening Algorithm. *Journal of Intellectual & Developmental Disability*, *32*(3), 179–87. doi:10.1080/13668250701604776

Witwer, A. N., & Lecavalier, L. (2008). Examining the validity of autism spectrum disorder subtypes. *Journal of Autism and Developmental Disorders*, *38*(9), 1611–24. doi:10.1007/s10803-008-0541-2

Zillmer, E. A., Spiers, M. V, & Culbertson, W. C. (2008). *Principles of Neuropsychology*. Belmont: Wadswprtj.

Appendix A

Demographic Survey

A. Child's Information:

1. Name: _____
2. School:_____
3. Age: _____
4. Date of Birth (yy/mm/dd): _____
5. Sex (circle one): Male Female
6. Ethnicity: White Black Indian Coloured
   Asian
   Other If other please specify: _____
7. Home Language: _____
8. Handedness (circle one): Left Right Ambidextrous
9. Number of siblings: _____
10. Number of **older** siblings: _____
11. How often does your child use a computer (circle one)?
    Never A few times a year Once a month Once a week
    Every day

12. Has your child ever experienced a head injury? (e.g., being hit on the head with an object and losing consciousness as a result) YES NO
    If yes, please give details: _____
    _____
    _____
    _____

13. Has your child ever experienced any of the following medical conditions:
    a. Neurological problems (e.g., epilepsy, meningitis, cerebral palsy, encephalitis, Tourette's syndrome, brain tumour) YES NO
       If yes, please specify: _____

    b. Depression YES NO
       If yes, please specify: _____

    c. Memory problems YES NO
       If yes, please specify: _____

    d. Problems with their vision: YES NO
       If yes, please specify: _____

e. Problems with their hearing YES NO
If yes, please specify: _____

f. Is he/she currently taking any prescription medication? YES NO
If yes, what medication(s)?
_____

14. Has your child ever been diagnosed with a social disorder, such as conduct disorder or oppositional defiant disorder (ODD)? YES NO
If yes, please specify:_____

15. Has your child ever had a communication disorder? (For example: Having problems with understanding or producing speech, slow vocabulary development, difficulties recalling words or problems with producing sentences appropriate for his/her age.) YES NO
If yes, please specify:_____

16. Has your child ever been diagnosed with a pervasive developmental disorder (PDD) such as autism, Asperger's syndrome, Rett's disorder or childhood disintegrative disorder?
(Tick the appropriate block).
No developmental disorder _____
Autism _____
Asperger's Syndrome _____
PDD – Not Otherwise Specified _____
Other (please specify):_____

17. Has your child ever experienced learning difficulties such as dyslexia or attention-deficit / hyperactivity disorder (ADD/ ADHD)? YES NO
If yes, please specify:_____

B. Parent Information:

1. What is the total yearly income of the household in which you live? (Tick the appropriate block):
[NOTE: This should be total household income, not personal income.]

0-35 000:_____	36 000-75 000:_____	76 000-125 000:_____	126 000-175 000:_____
176 000-225 000:_____	226 000-275 000:_____	276 000-32 5000:_____	326 000-375 000:_____
376 000-425 000:_____	426 000-475 000:_____	476 000-525 000:_____	more than 526000:____

2. Highest level of education reached for mother, father and/or guardian (please circle appropriate number).

	Biological mother	Biological father	Guardian
1) 0 years (No Grades / Standards) = Never went to school	1	1	1
2) 1-6 years (Grades 1-6 / Sub A-Std 4) = Didn't complete primary school	2	2	2
3) 7 years (Grade 7 / Std 5) = Completed primary school	3	3	3
4) 8-11 years (Grades 8-11 / Stds 6-9) = Some secondary education (didn't complete high school)	4	4	4
5) 12 years (Grade 12 / Std 10) = Completed high school	5	5	5
6) 13+ years = Tertiary education Completed university / technikon / college	6	6	6
7) Don't know	7	7	7

3. Parental employment: (Please circle appropriate number)

	Biological mother	Biological father	Guardian
1. Higher executives, major professionals, owners of large businesses	1	1	1
2. Business managers of medium sized businesses, lesser professions (e.g. nurses, opticians, pharmacists, social workers, teachers)	2	2	2
3. Administrative personnel, managers, minor professionals, owners / proprietors of small businesses (e.g. bakery, car dealership, engraving business, plumbing business, florist, decorator, actor, reporter, travel agent)	3	3	3
4. Clerical and sales, technicians, small businesses (e.g. bank teller, bookkeeper, clerk, draftsperson, timekeeper, secretary)	4	4	4
5. Skilled manual – usually having had training (e.g. baker, barber, chef, electrician, fireman, machinist, mechanic, painter, welder, police, plumber, electrician)	5	5	5
6. Semi-skilled (e.g. hospital aide, painter, bartender, bus driver, cook, garage guard, checker, waiter, machine operator)	6	6	6
7. Unskilled (e.g. attendant, janitor, construction helper, unspecified labour, porter, unemployed)	7	7	7
8. Homemaker	8	8	8
9. Student, disabled, no occupation	9	9	9

4. Material and financial resources (please circle appropriate number).

Which of the following items, in working order, does your household have?

Items	Yes	No
1. A refrigerator or freezer	1	1
2. A vacuum cleaner or polisher	2	2
3. A television	3	3
4. A hi-fi or music center (radio excluded)	4	4
5. A microwave oven	5	5
6. A washing machine	6	6
7. A video cassette recorder or dvd player	7	7

Which of the following do you have in your home?

Items	Yes	No
1. Running water	1	1
2. A domestic servant	2	2
3. At least one car	3	3
4. A flush toilet	4	4
5. A built-in kitchen sink	5	5
6. An electric stove or hotplate	6	6
7. A working telephone	7	7

Do you personally do any of the following?

Items	Yes	No
1. Shop at supermarkets	1	1
2. Use any financial services such as a bank account, ATM card or credit card	2	2
3. Have an account or credit card at a retail store	3	3

Appendix B

Parental Consent

An Exploration of the Relationships between Autism Spectrum Disorders, Theory of Mind, and the Serotonin Transporter Promoter Length Polymorphism

Principal Researchers: Dr Susan Malcolm-Smith
Dr Colleen O'Ryan

You and your child are invited to participate in a study investigating the relationships between Autism Spectrum Disorders, Theory of Mind abilities, and a gene that may be involved in Autism Spectrum Disorders. Theory of Mind is a skill that is needed for social interaction, and many children with Autism Spectrum Disorders do not fully develop this ability. We would therefore like to see how this ability is related to different symptoms, and then to see what role this gene plays in symptoms and in Theory of Mind.

This study will recruit approximately 100 children between 8 and 14 years old who have current Autism Spectrum Disorder diagnoses. You, another caregiver, or a teacher may be present during the assessment. Assessment will take between 15 and 90 minutes depending on which tasks your child is asked to complete.

I will start the assessment by collecting a cheek swab in order to get a DNA sample. I will take a cotton bud and gently rub it on the inside of your child's cheek. To make sure your child is comfortable, I will first let them play with a cotton bud and get used to putting it in their mouth. They can then imitate me showing them how to rub the inside of the cheek. Once your child is comfortable, I will collect the sample.

I will then ask your child to play a game that involves pointing to different pictures of bunnies. This will assess their ability to follow two-stage commands (for instance, "Show me the happy, blue bunny"). Some children will then be asked to complete Theory of Mind tasks. These tasks are all toy or story based tests for social perception and social cognition.

You will be asked to complete a demographics survey, as well as three questionnaires that rate your child's symptoms. The demographic survey asks question about your child's medical history and about your household so that we can better understand them. These are all

short, pen and paper surveys where you reply to a question with either a 'yes/no' answer, or rate the truth of the statement on a 4-point scale.

There is no risk involved in participating in this study. If you or your child feel uncomfortable at any time you may withdraw from the study without any negative consequences. We will take strict precautions to ensure that your personal information is kept safe and confidential. We will not use any personal identifiers on any of the information, but will instead use codes, and all data will be kept in a locked file cabinet or a password-protected, secure computer. The cheek swabs will be securely frozen and stored at the University of Cape Town's Department of Molecular and Cell Biology. If data from this study leads to publication, your child's contributions will be kept anonymous.

If you have any questions or queries about the research or your participation, please do not hesitate to contact Dr Susan Malcolm-Smith, myself, or the Psychology Department's Ethics Committee at the University of Cape Town.

Dr Susan Malcolm-Smith
Lecturer
Department of Psychology
University of Cape Town
021-650-4605
susan.malcolm-smith@uct.ac.za

Dr Colleen O'Ryan
Lecturer
Department of Molecular and Cell Biology
University of Cape Town
021-650-4257
colleen.oryan@uct.ac.za

Katie Hamilton
MA Neuropsychology Candidate
Department of Psychology
University of Cape Town
082 463 8335
kate@hamilton.co.za

Rosalind Adams
Administration Assistant: Ethics Committee
Department of Psychology
University of Cape Town
021-650-4104
rosalind.adams@uct.ac.za

Dear Parent(s),

Thank you for taking part in our study!

If you would like your child to participate in the study, please sign the consent form and complete the demographic questionnaire provided. This information is necessary to identify any possible conditions that would exclude your child from being able to take part in the study and to identify possible factors that could influence their symptoms or their theory of mind development in any way. We also require some information about your child's medical history so that we can better understand your child. Therefore please answer all questions as accurately and truthfully as possible. Once we have established which children will be able to participate in the study, we will send you symptom questionnaires and further demographic questions to please complete.

We understand that some of this information may be sensitive, but please be assured that all information will be kept strictly confidential. Neither you nor your child will be discriminated against, or lose any privileges, as a result of information given. Only certain authorized researchers at UCT will be able to view the information. The information will then be saved as part of a dataset which may only include information that cannot directly identify you or your child. For example, the dataset may not include you or your child's name, address, telephone number, ID number or any other photographs, numbers, codes or so forth that link you or your child to the study. If the results of the research are published neither you nor your child will be identified in any way.

If you have any queries or concerns please feel free to contact us.

Thank you for your participation.

Katie Hamilton
Department of Psychology
University of Cape Town
Cell: 082 463 8335
Email: kate@hamilton.co.za

Consent Form

The study has been explained to me, and my questions have been answered. I understand that participation in this study is voluntary, and that I may withdraw my child at any point. I understand that my child will not be identified except by an initial, and that this anonymity will be maintained throughout the study and when the research is published.
I consent to allow my child to participate in this study.

Child's name _____

Signature of parent/guardian _____

Date _____

I hereby give consent for DNA samples to be collected from my child using cheek swabs. I understand that this DNA will only be used for research purposes. I give consent for this DNA to be stored at the Department of Molecular and Cell Biology, UCT, and to be used in later research.

Child's name _____

Signature of parent/guardian _____

Date _____

{Parent/guardian} _____ has been informed of the purpose, procedures, and any possible risks or this study. He / she has been given time to ask any questions, and these questions have been answered to the best of my ability. He / she understands that participation is voluntary.

Researcher _____

Signature _____

Date _____

Please indicate below if you would like to be notified of future research projects conducted by our research group:
Yes, I _____ (initial) would like to be added to your research participation pool and be notified of research projects in which I or my child might participate in the future.

Method of contact:

Phone number: _____

Cell phone number: _____

E-mail address: _____

Mailing address: _____

Appendix C

Autism Social Skills Profile

Child's name: _____

Date of birth: _____ Today's Date: _____

Your name:_____

Your relationship to the child: _____

The following phrases describe skills or behaviours that your child might exhibit during social interactions or social situations. Please rate **HOW OFTEN** your child exhibits each skill or behaviour independently, without assistance from others (i.e. without reminders, cueing and/or prompting). You should base your judgement on your child's behaviour **OVER THE LAST THREE MONTHS**.

Please do not skip any items. If you are unsure of an item, please provide your best estimate. You may use the "Brief Description" section to provide additional information on the particular skill or behaviour. For instance, if your child will exhibit a particular skill or behaviour more frequently when cueing or prompting is provided, or when interacting with adults rather than peers, please make note of this in the "Brief Description" section.

Please use the following guidelines to rate your child's behaviour:

Never	:	Your child never or almost never exhibits the skill of behaviour
Sometimes	:	You child sometimes or occasionally exhibits the skill or behaviour
Often	:	Your child often or typically exhibits the skill or behaviour
Very often	:	You child very often or always exhibits the skill of behaviour

	SKILL AREA	HOW OFTEN	BRIEF DESCRIPTION
1	Invites peers to join him/her in activities	☐Never ☐Sometimes ☐Often ☐Very Often	
2	Joins activities with peers	☐Never ☐Sometimes ☐Often ☐Very Often	
3	Takes turns during games and activities	☐Never ☐Sometimes ☐Often ☐Very Often	
4	Maintains person hygiene	☐Never ☐Sometimes ☐Often ☐Very Often	
5	Interacts with peers during unstructured activities	☐Never ☐Sometimes ☐Often ☐Very Often	
6	Interacts with peers during structured activities	☐Never ☐Sometimes ☐Often ☐Very Often	
7	Asks questions to request information about a person	☐Never ☐Sometimes ☐Often ☐Very Often	
8	Asks questions to request information about a topic	☐Never ☐Sometimes ☐Often ☐Very Often	
9	Engages in one-on-one social interactions with peers	☐Never ☐Sometimes ☐Often ☐Very Often	
10	Interacts with groups of peers	☐Never ☐Sometimes ☐Often ☐Very Often	
11	Maintains the “give-and-take” of conversations	☐Never ☐Sometimes ☐Often ☐Very Often	
12	Expresses sympathy of others	☐Never ☐Sometimes ☐Often ☐Very Often	
13	Talks about or acknowledges the interests of others	☐Never ☐Sometimes ☐Often ☐Very Often	
14	Recognises the facial expressions of others	☐Never ☐Sometimes ☐Often ☐Very Often	
15	Recognises the nonverbal cues or body language of others	☐Never ☐Sometimes ☐Often ☐Very Often	
16	Requests assistance from others	☐Never ☐Sometimes ☐Often ☐Very Often	
17	Understands the jokes or humour of others	☐Never ☐Sometimes ☐Often ☐Very Often	

18	Maintains eye contact during conversations	☐Never ☐Sometimes ☐Often ☐Very Often	
19	Maintains an appropriate distance when interacting with peers	☐Never ☐Sometimes ☐Often ☐Very Often	
20	Speaks with an appropriate volume in conversations	☐Never ☐Sometimes ☐Often ☐Very Often	
21	Considers multiple viewpoints	☐Never ☐Sometimes ☐Often ☐Very Often	
22	Offers assistance to others	☐Never ☐Sometimes ☐Often ☐Very Often	
23	Verbally expresses how he/she is feeling	☐Never ☐Sometimes ☐Often ☐Very Often	
24	Responds to the greetings of others	☐Never ☐Sometimes ☐Often ☐Very Often	
25	Joins a conversation with two or more people without interrupting	☐Never ☐Sometimes ☐Often ☐Very Often	
26	Initiates greetings with others	☐Never ☐Sometimes ☐Often ☐Very Often	
27	Provides compliments to others	☐Never ☐Sometimes ☐Often ☐Very Often	
28	Introduces self to others	☐Never ☐Sometimes ☐Often ☐Very Often	
29	Politely asks others to move out of his/her way	☐Never ☐Sometimes ☐Often ☐Very Often	
30	Acknowledges the compliments directed at him/her in activities	☐Never ☐Sometimes ☐Often ☐Very Often	
31	Allows peers to join him/her in activities	☐Never ☐Sometimes ☐Often ☐Very Often	
32	Responds to the invitations of peers to join them in activities	☐Never ☐Sometimes ☐Often ☐Very Often	
33	Allows other to assist him/her with tasks	☐Never ☐Sometimes ☐Often ☐Very Often	

34	Responds to questions directed at him/her by others	☐Never ☐Sometimes ☐Often ☐Very Often	
35	Experiences positive peer interactions	☐Never ☐Sometimes ☐Often ☐Very Often	
36	Compromises during disagreements with others	☐Never ☐Sometimes ☐Often ☐Very Often	
37	Responds slowly in conversation	☐Never ☐Sometimes ☐Often ☐Very Often	
38	Changes the topic of conversation to fit self-interests	☐Never ☐Sometimes ☐Often ☐Very Often	
39	Misinterprets the intentions of others	☐Never ☐Sometimes ☐Often ☐Very Often	
40	Makes inappropriate comments	☐Never ☐Sometimes ☐Often ☐Very Often	
41	Engages in solitary interests and hobbies	☐Never ☐Sometimes ☐Often ☐Very Often	
42	Ends conversations abruptly	☐Never ☐Sometimes ☐Often ☐Very Often	
43	Fails to read cues to terminate conversations	☐Never ☐Sometimes ☐Often ☐Very Often	
44	Exhibits fear or anxiety regarding social interactions	☐Never ☐Sometimes ☐Often ☐Very Often	
45	Experiences negative peer interactions	☐Never ☐Sometimes ☐Often ☐Very Often	
46	Engages in socially inappropriate behaviour	☐Never ☐Sometimes ☐Often ☐Very Often	
47	Exhibits poor timing with his/her social initiations	☐Never ☐Sometimes ☐Often ☐Very Often	
48	Is manipulated by peers	☐Never ☐Sometimes ☐Often ☐Very Often	
49	Engages in solitary activity in the presence of others	☐Never ☐Sometimes ☐Often ☐Very Often	

Appendix D

Social Communication Questionnaire – Lifetime Form

Child's name: _____

Date of birth: _____ Today's Date:_____

Your name: _____

Your relationship to the child: _____

Thank you for taking the time to complete this questionnaire. This questionnaire focuses on the behaviour of your child during their lifetime. Please answer each question with a *yes* or a *no*. A few questions ask about several related types of behaviour; please answer *yes* if any of these behaviours have ever been present. Although you may be uncertain about whether some behaviours were ever present or not, please answer *yes* or *no* to every question on the basis of what you think.

1. Is she/he now able to talk using short phrases or sentences? a. If no skip to question 8.	YES / NO
2. Can you have a to and fro "conversation" with her/him that involves taking turns or building on what you have said?	YES / NO
3. Has she/he ever used odd phrases or said the same thing over and over in almost exactly the same way (either phrases that she/he hears other people use or ones that she/he makes up)?	YES / NO
4. Has she/he ever used socially inappropriate questions or statements? For example, has she/he ever regularly asked personal questions or made personal comments at awkward times?	YES / NO
5. Has she/he ever got her/his pronouns mixed up (e.g. saying you or she/he for I)?	YES / NO
6. Has she/he ever used words that she/he seemed to have invented or made up her/himself put things in odd, indirect ways; or used metaphorical ways of saying things (e.g. saying *hot rain* for *steam*)?	YES / NO

7. Has she/he ever said the same thing over and over in exactly the same way or insisted that you say the same thing over and over again?	YES / NO
8. Has she/he ever had things that she/he seemed to have to do in a very particular way or order, or rituals that she/he insisted that you go through?	YES / NO
9. Has her/his facial expression usually seemed appropriate to the particular situation, as far as you could tell?	YES / NO
10. Has she/he ever used your hand like a tool or as if it were a part of her/his own body (e.g. pointing with your finger or putting your hand on a doorknob to get you to open the door)?	YES / NO
11. Has she/he had any interests that preoccupy her/him and might seem odd to other people (e.g. traffic lights, drainpipes or timetables)?	YES / NO
12. Has she/he ever seemed to be more interested in parts of a toy or an object (e.g. spinning the wheels of a car), rather than in using the object as it was intended?	YES / NO
13. Has she/he ever had any special interests that are *unusual* in their intensity, but otherwise appropriate for her/his age and peer group (e.g. trains or dinosaurs)?	YES / NO
14. Has she/he ever seemed to be *unusually* interested in the sight, feel, sound, taste, or smell of things or people?	YES / NO
15. Has she/he ever had any mannerism or odd ways of moving her/his hands or fingers, such as flapping or moving her/his fingers in front of her/his eyes?	YES / NO
16. Has she/he ever had any complicated movements of her/his whole body, such as spinning or repeatedly bouncing up and down?	YES / NO
17. Has she/he ever injured her/himself deliberately, such as by biting her/his arm or banging her/his head?	YES / NO
18. Has she/he ever had any objects (*other* than a soft toy or comfort blanket) that she/he *had* to carry around?	YES / NO
19. Does she/he have any particular friends or a best friend?	YES / NO

For the following behaviours, please focus on the time period between the child's fourth and fifth birthdays. You may find it easier to remember how things were at that time by focusing on key events, such as starting school, moving house, Christmastime, or other specific events that are particularly memorable for you as a family.

20. When she/he was 4 to 5, did she/he ever talk with you just to be friendly (rather than to get something)?	YES / NO
21. When she/he was 4 to 5, did she/he ever *spontaneously* copy you (or other people) or what you were doing (such as vacuuming, gardening or mending things)?	YES / NO
22. When she/he was 4 to 5, did she/he ever spontaneously point at things around her/him just to show you things (not because she/he wanted them)?	YES / NO
23. When she/he was 4 to 5, did she/he ever use gestures, other than pointing or pulling your hand, to let you know what she/he wanted?	YES / NO
24. When she/he was 4 to 5, did she/he nod her/his head to mean *yes*?	YES / NO
25. When she/he was 4 to 5, did she/he shake her/his head to mean *no*?	YES / NO
26. When she/he was 4 to 5, did she/he usually look at you directly in the face when doing things with you or talking with you?	YES / NO
27. When she/he was 4 to 5, did she/he smile back if someone smiled at her/him?	YES / NO
28. When she/he was 4 to 5, did she/he ever show you things that interested him/her to engage your attention?	YES / NO
29. When she/he was 4 to 5, did she/he ever offer to share things other than food with you?	YES / NO
30. When she/he was 4 to 5, did she/he ever seem to want you to join in her/his enjoyment of something?	YES / NO
31. When she/he was 4 to 5, did she/he ever try to comfort you if you were sad or hurt?	YES / NO

32. When she/he was 4 to 5, when she/he wanted something or wanted help, did she/he look at you and use gestures with sounds or word to get your attention?	YES / NO
33. When she/he was 4 to 5, did she/he show a normal range of facial expressions?	YES / NO
34. When she/he was 4 to 5, did she/he ever spontaneously join in and try to copy the actions in social games, such as *Ring of Roses, 123 Stop, Stuck in the Mud,* or *Mamba*?	YES / NO
35. When she/he was 4 to 5, did she/he play any pretend or make-believe games?	YES / NO
36. When she/he was 4 to 5, did she/he seem interested in other children of approximately the same age whom she/he did not know?	YES / NO
37. When she/he was 4 to 5, did she/he respond positively when another child approached her/him?	YES / NO
38. When she/he was 4 to 5, if you came into a room and started talking to her/him without calling her/his name, did she/he usually look up and pay attention to you?	YES / NO
39. When she/he was 4 to 5, did she/he ever play imaginative games with another child in such a way that you could tell that each child understood what the other was pretending?	YES / NO
40. When she/he was 4 to 5, did she/he play cooperatively in games that required joining in with a group of other children, such as hide-and-seek or ball games?	YES / NO

Appendix E

Restricted and Repetitive Behavior Scale – Revised

Please rate your child's behaviour by reading each of the items listed and then choosing the score that best represents the frequency of this behaviour for your child. Make your ratings based on your observations and interactions with the person over the last month. Use the definitions in the box given below to score each item:

0 =	behaviour does not occur
1 =	mild presentation, does not cause a problem for the child
2 =	moderate presentation, sometimes causes problems for the child
3 =	severe presentation, almost always causes problems for the child

I. Stereotyped Behaviour Subscale

Definition: apparently purposeless movements or actions that are repeated in a similar manner

		Does not occur	*Mild presentation*	*Moderate presentation*	*Severe presentation*
1	WHOLE BODY (body rocking, body swaying)	0	1	2	3
2	HEAD (rolls head, nods head, turns head)	0	1	2	3
3	HAND/FINGER (flaps hands, wiggles or flicks fingers, claps hands, waves or shakes hand or arm)	0	1	2	3
4	LOCOMOTION (turns in circles, whirls, jumps, bounces)	0	1	2	3
5	OBJECT USAGE (spins or twirls objects, twiddles or slaps or throws objects, lets objects fall out of hands)	0	1	2	3
6	SENSORY (covers eyes, looks closely or gazes at hands or objects, covers ears, smells or sniffs items, rubs surfaces)	0	1	2	3

How <u>often</u> do they happen?
(If never, then skip to Section II)

Never — Constantly

How <u>upset</u> does your child get when interrupted?

Not at all — Extremely

How much do these behaviours <u>get in the way of ongoing events?</u>

Not at all — Severe Interference

II. Self-injurious Behaviour Subscale

Definition: movement or actions that have the potential to cause redness, bruising, or other injury to the body, and that are repeated in a similar manner

		Does not occur	*Mild presentation*	*Moderate presentation*	*Severe presentation*
7	HITS SELF WITH BODY PART (hits or slaps head, face, or other body area)	0	1	2	3
8	HITS SELF AGAINST SURFACE OR OBJECT (hits or bangs head or other body part on table, floor or other surface)	0	1	2	3
9	HITS SELF WITH OBJECT (hits or bangs head or other body area with objects)	0	1	2	3
10	BITES SELF (bites hand, wrist, arm, lips or tongue)	0	1	2	3
11	PULLS (pulls hair or skin)	0	1	2	3
12	RUBS OR SCRATCHES SELF (rubs or scratches marks on arms, leg, face or torso)	0	1	2	3
13	INSERTS FINGERS OR OBJECTS (eye-poking, ear-poking)	0	1	2	3
14	SKIN PICKING (picks at skin on face, hands, arms, legs or torso)	0	1	2	3

How <u>often</u> do they happen?
(If never, then skip to Section III)

How <u>upset</u> does your child get when interrupted?

How much do these behaviours <u>get in the way of ongoing events?</u>

0 =	behaviour does <u>not</u> occur
1 =	mild presentation, does not cause a problem for the child
2 =	moderate presentation, sometimes causes problems for the child
3 =	severe presentation, almost always causes problems for the child

III. Compulsive Behaviour Subscale

Definition: behaviour that is repeated and is performed according to a rule, or involves things being done "just so"

		Does not occur	*Mild presentation*	*Moderate presentation*	*Severe presentation*
15	ARRANGING/ORDERING (arranges certain objects in a particular pattern or place; need for things to be even or symmetrical)	0	1	2	3
16	COMPLETENESS (must have doors opened or closed; takes all items out of a container or area)	0	1	2	3
17	WASHING/CLEANING (excessively cleans certain body parts; picks at link or loose threads)	0	1	2	3
18	CHECKING (repeatedly checks doors, windows, drawers, appliances, clocks, locks, etc)	0	1	2	3
19	COUNTING (counts items or objects; counts to a certain number or in a certain way)	0	1	2	3
20	HOARDING/SAVING (collects, hoards or hides specific items)	0	1	2	3
21	REPEATING (need to repeat routine events; in/out door, up/down from chair, clothing on/off)	0	1	2	3
22	TOUCH/TAP (need to touch, tap, or rub items, surfaces, or people)	0	1	2	3

How <u>often</u> do they happen?
(If never, then skip to Section IV)

How <u>upset</u> does your child get when interrupted?

How much do these behaviours <u>get in the way of ongoing events?</u>

0 =	behaviour does <u>not</u> occur
1 =	mild presentation, does not cause a problem for the child
2 =	moderate presentation, sometimes causes problems for the child
3 =	severe presentation, almost always causes problems for the child

IV. Ritualistic Behaviour Subscale

Definition: performing activities of daily living in a similar manner

		Does not occur	*Mild presentation*	*Moderate presentation*	*Severe presentation*
23	EATING/MEALTIME (strongly prefers/insists on eating/drinking only certain things; eats or drinks items in a set order; insists that meal related items are arranged in a certain way)	0	1	2	3
24	SLEEPING/BEDTIME (insists on certain pre-bedtime routines; arranges items in room "just so" prior to bedtime; insists that certain items be present with him/her during sleep; insists that another person be present prior to or during sleep)	0	1	2	3
25	SELF-CARE – BATHROOM AND DRESSING (insists on specific order of activities or tasks related to using the bathroom, to washing, showering, bathing or dressing; arranges items in a certain way in the bathroom or insists that bathroom items not be moved; insists on wearing certain clothing items)	0	1	2	3
26	TRAVEL/TRANSPORTATION (insists on taking certain routes/paths; must sit in specific location in vehicles; insists that certain items be present during travel, e.g. toy or material; insists on seeing or touching certain things or places during travel, such as a sign or store)	0	1	2	3
27	PLAY/LEISURE (insists on certain play activities; follows a rigid routine during play/leisure; insists that certain items be present/available during play/leisure; insists that other persons do certain things during play)	0	1	2	3
28	COMMUNICATION/SOCIAL INTERACTIONS (repeats same topic(s) during social interactions; repetitive questioning; insists on certain topics of conversation; insists that others say certain things or respond in certain ways during interactions)	0	1	2	3

How <u>often</u> do they happen?
(If never, then skip to Section V)

How <u>upset</u> does your child get when interrupted?

How much do these behaviours get in the way of ongoing events?

V. Sameness Behaviour Subscale

Definition: resistance to change, insisting that things stay the same

		Does not occur	*Mild presentation*	*Moderate presentation*	*Severe presentation*
29	Insists that things remain in the same place(s) (e.g. toys, supplies, furniture, pictures, etc)	0	1	2	3
30	Objects to / resists visiting new places	0	1	2	3
31	Becomes upset if interrupted in what he/she is doing	0	1	2	3
32	Insists on walking in a particular pattern (e.g. straight line)	0	1	2	3
33	Insists on sitting at the same place	0	1	2	3
34	Disliked changes in appearance or behaviour of the people around him/her	0	1	2	3
35	Insists on using a particular door	0	1	2	3
36	Likes the same CD, take, record or piece of music played continually; likes same movie/video or part of movie/video	0	1	2	3
37	Resists changing activities; difficulty with transitions	0	1	2	3
38	Insists on same routine, household, school or work schedule everyday	0	1	2	3
39	Insists that specific things take place as specific times	0	1	2	3

How often do they happen?
(If never, then skip to Section VI)

How upset does your child get when interrupted?

How much do these behaviours get in the way of ongoing events?

VI. Restricted Behaviour Subscale

Definition: limited range of focus, interest or activity

		Does not occur	*Mild presentation*	*Moderate presentation*	*Severe presentation*
40	Fascination, preoccupation with one subject or activity (e.g. trains, computers, weather, dinosaurs)	0	1	2	3
41	Strongly attached to one specific object	0	1	2	3
42	Preoccupation with part(s) of objects rather than the whole object (e.g. buttons on clothes, wheels on toy cars)	0	1	2	3
43	Fascination, preoccupation with movement / things that move (e.g. fans, clocks)	0	1	2	3

How <u>often</u> do they happen?
(If never, then skip to Section VII)

Never — Constantly

How <u>upset</u> does your child get when interrupted?

Not at all — Extremely

How much do these behaviours <u>get in the way of ongoing events?</u>

Not at all — Severe Interference

VII. Final Question

Overall, if you "lump together" all of the behaviours described in this questionnaire, how much of a problem are these repetitive behaviours (both for the person with autism, as well as how they affect the people around them)? please rate on a scale of 1 to 100, where 1 = "not a problem" and 100 = "as bad as you can imagine":

Score from 1-100: _____

0 =	behaviour does <u>not</u> occur
1 =	mild presentation, does not cause a problem for the child
2 =	moderate presentation, sometimes causes problems for the child
3 =	severe presentation, almost always causes problems for the child

Appendix F

Assent Form

Hello! We want to tell you about a research study we are doing. A research study is a way to learn more about something, and we want to learn more about autism!

If you join this study, I will ask you to put a cotton bud inside your mouth and rub your cheek with it. This will not hurt you at all. I then ask you to play a game with me where I ask you to point to some pictures. I might then ask you to listen to a few stories and look at some pictures. I will then ask you some questions about the stories. You might also be asked to do tasks like playing with some toys or stickers. If you get tired, then we can take a break. You can bring your parent or guardian with if you want to.

You do not have to join this study. It is up to you. No one will get upset if you don't want to be in the study. You won't get into trouble if you don't join this study. It is also fine if you join the study, but then change your mind and want to stop. You can decide at any time to stop being in this study.

Do you have any questions?

{Participant's name} _____ has been informed of the purpose, procedures, and any possible risks or this study. He / she has been given time to ask any questions, and these questions have been answered to the best of my ability. He / she understands that participation is voluntary.

Researcher _____

Signature _____

Date _____

Appendix G

Weak Items Removed from the ASSP

7. Asks questions to request information about a person
8. Asks questions to request information about a topic
16. Requests assistance from others
33. Allows other to assist him/her with tasks
37. Responds slowly in conversation
38. Changes the topic of conversation to fit self-interests
39. Misinterprets the intentions of others
40. Makes inappropriate comments
42. Ends conversations abruptly
43. Fails to read cues to terminate conversations
45. Experiences negative peer interactions
48. Is manipulated by peers

Appendix H

Factor loadings based on principal component analysis with varimax rotation for the ASSP for a forced three factor solution to assess the standard ASSP subscales after weak items were removed

Factors 1 – 3 show the factor loading that emerged when a forced 3 factor model was run with principal component factor analysis for the ASSP. As the ASSP has three standard subscales, these are shown to compare how items loaded in our sample compared to the model that was expected based on the reported ASSP structure.

	Factor 1	Factor 2	Factor 3	ASSP Subscale
1. Invites peers to join him/her in activities	**.59**	.28	.32	Social Participation / Avoidance
2. Joins activities with peers	**.73**	.26		Social Participation / Avoidance
3. Takes turns during games and activities	**.52**	.26		Social Reciprocity
4. Maintains person hygiene		.31		Social Reciprocity
5. Interacts with peers during unstructured activities	**.76**			Social Participation / Avoidance
6. Interacts with peers during structured activities	**.75**			Social Participation / Avoidance
9. Engages in one-on-one social interactions with peers	**.66**	.27		Social Participation / Avoidance
10. Interacts with groups of peers	**.66**	.32		Social Participation / Avoidance
11. Maintains the "give-and-take" of conversations		.43	.43	Social Reciprocity
12. Expresses sympathy for others		**.55**	**.54**	Social Reciprocity
13. Talks about or acknowledges the interests of others		.45	**.55**	Social Reciprocity
14. Recognises the facial expressions of others		.26	**.61**	Social Reciprocity
15. Recognises the nonverbal cues or body language of others			**.72**	Detrimental Social Behaviours
17. Understands the jokes or humour of others	.33		.44	Social Reciprocity
18. Maintains eye contact during conversations	.28		.26	Detrimental Social Behaviours
19. Maintains an appropriate distance when interacting with peers				Social Reciprocity
20. Speaks with an appropriate volume in conversations	.41	.38		Social Reciprocity

21. Considers multiple viewpoints		.42	.43	Social Reciprocity
22. Offers assistance to others	.26	**.73**		Social Reciprocity
23. Verbally expresses how he/she is feeling		**.66**		Social Reciprocity
24. Responds to the greetings of others		**.68**		Social Reciprocity
25. Joins a conversation with two or more people without interrupting	.36	.30		Social Reciprocity
26. Initiates greetings with others		**.74**		Social Reciprocity
27. Provides compliments to others		**.60**	.42	Social Reciprocity
28. Introduces self to others		**.75**		Social Reciprocity
29. Politely asks others to move out of his/her way		**.58**	.31	Social Reciprocity
30. Acknowledges the compliments directed at him/her in activities		.40		Social Reciprocity
31. Allows peers to join him/her in activities	**.58**		.25	Social Participation / Avoidance
32. Responds to the invitations of peers to join them in activities	**.77**			Social Participation / Avoidance
34. Responds to questions directed at him/her by others	.45	.39		Social Reciprocity
35. Experiences positive peer interactions	**.67**	.36		Social Participation / Avoidance
36. Compromises during disagreements with others	**.56**		.33	Social Reciprocity
41. Engages in solitary interests and hobbies	.48		.53	Social Participation / Avoidance
44. Exhibits fear or anxiety regarding social interactions	**.67**			Social Participation / Avoidance
46. Engages in socially inappropriate behaviour	**.63**		.35	Detrimental Social Behaviours
47. Exhibits poor timing with his/her social initiations	**.63**		.27	Detrimental Social Behaviours
49. Engages in solitary activity in the presence of others	**.62**		**.58**	Social Participation / Avoidance

Note: Factor loadings below .25 are suppressed

Appendix I

Weak Items Removed from the SCQ-Lifetime

---

1. Is she/he now able to talk using short phrases or sentences? If no skip to question 8.

2. Can you have a to and fro "conversation" with her/him that involves taking turns or building on what you have said?

4. Has she/he ever used socially inappropriate questions or statements? For example, has she/he ever regularly asked personal questions or made personal comments at awkward times?

6. Has she/he ever used words that she/he seemed to have invented or made up her/himself put things in odd, indirect ways; or used metaphorical ways of saying things (e.g. saying *hot rain* for *steam*)?

11. Has she/he had any interests that preoccupy her/him and might seem odd to other people (e.g. traffic lights, drainpipes or timetables)?

13. Has she/he ever had any special interests that are *unusual* in their intensity, but otherwise appropriate for her/his age and peer group (e.g. trains or dinosaurs)?

15. Has she/he ever had any mannerism or odd ways of moving her/his hands or fingers, such as flapping or moving her/his fingers in front of her/his eyes?

17. Has she/he ever injured her/himself deliberately, such as by biting her/his arm or banging her/his head?

18. Has she/he ever had any objects (*other* than a soft toy or comfort blanket) that she/he *had* to carry around?

19. Does she/he have any particular friends or a best friend?

21. When she/he was 4 to 5, did she/he ever *spontaneously* copy you (or other people) or what you were doing (such as vacuuming, gardening or mending things)?

32. When she/he was 4 to 5, when she/he wanted something or wanted help, did she/he look at you and use gestures with sounds or word to get your attention?

34. When she/he was 4 to 5, did she/he ever spontaneously join in and try to copy the actions in social games, such as *Ring of Roses, 123 Stop, Stuck in the Mud,* or *Mamba*?

35. When she/he was 4 to 5, did she/he play any pretend or make-believe games?

Appendix J

Factor loadings based on principal component analysis with varimax rotation for the SCQ for a forced four factor solution to assess the standard SCQ subscales after weak items were removed

Factors 1 – 4 show the factor loading that emerged when a forced 4 factor model was run with principal component factor analysis for the SCQ. As the SCQ has four standard subscales, these are shown to compare how items loaded in our sample compared to the model that was expected based on the reported SCQ structure.

	Factor 1	Factor 2	Factor 3	Factor 4	SCQ Subscales
3. Has she/he ever used odd phrases or said the same thing over and over in almost exactly the same way (either phrases that she/he hears other people use or ones that she/he makes up)?		.41	.48		Communication
5. Has she/he ever got her/his pronouns mixed up (e.g. saying you or she/he for I)?	-.34	.48	.33	.40	Abnormal Language
7. Has she/he ever said the same thing over and over in exactly the same way or insisted that you say the same thing over and over again?		.42			Abnormal Language
8. Has she/he ever had things that she/he seemed to have to do in a very particular way or order, or rituals that she/he insisted that you go through?			.30	**.69**	Abnormal Language
9. Has her/his facial expression usually seemed appropriate to the particular situation, as far as you could tell?	.37	.33	-.28		Stereotyped Behaviours
10. Has she/he ever used your hand like a tool or as if it were a part of her/his own body (e.g. pointing with your finger or putting your hand on a doorknob to get you to open the door)?			.38	.47	Communication
12. Has she/he ever seemed to be more interested in parts of a toy or an object (e.g. spinning the wheels of a car), rather than				**.66**	Stereotyped Behaviours

in using the object as it was intended?					
14. Has she/he ever seemed to be unusually interested in the sight, feel, sound, taste, or smell of things or people?	.34	.45		.33	Stereotyped Behaviours
16. Has she/he ever had any complicated movements of her/his whole body, such as spinning or repeatedly bouncing up and down?				**.74**	Communication
20. When she/he was 4 to 5, did she/he ever talk with you just to be friendly (rather than to get something)?	**.52**	.33	.27		Social Interaction
22. When she/he was 4 to 5, did she/he ever spontaneously point at things around her/him just to show you things (not because she/he wanted them)?		**.60**			Social Interaction
23. When she/he was 4 to 5, did she/he ever use gestures, other than pointing or pulling your hand, to let you know what she/he wanted?		**.58**			Social Interaction
24. When she/he was 4 to 5, did she/he nod her/his head to mean *yes*?		**.70**			Communication
25. When she/he was 4 to 5, did she/he shake her/his head to mean *no*?		**.73**			Communication
26. When she/he was 4 to 5, did she/he usually look at you directly in the face when doing things with you or talking with you?	.39			.49	Social Interaction
27. When she/he was 4 to 5, did she/he smile back if someone smiled at her/him?	**.69**				Social Interaction
28. When she/he was 4 to 5, did she/he ever show you things that interested him/her to engage your attention?	.29				Social Interaction
29. When she/he was 4 to 5, did she/he ever offer to share things other than food with you?	**.55**				Social Interaction
30. When she/he was 4 to 5, did she/he ever seem to want you to join in her/his enjoyment of something?	**.69**				Social Interaction

31. When she/he was 4 to 5, did she/he ever try to comfort you if you were sad or hurt?	**.73**			Social Interaction
33. When she/he was 4 to 5, did she/he show a normal range of facial expressions?	**.60**			Social Interaction
36. When she/he was 4 to 5, did she/he seem interested in other children of approximately the same age whom she/he did not know?	**.61**	.28		Social Interaction
37. When she/he was 4 to 5, did she/he respond positively when another child approached her/him?	.46	**.61**	.27	Social Interaction
38. When she/he was 4 to 5, if you came into a room and started talking to her/him without calling her/his name, did she/he usually look up and pay attention to you?	.32	.46	.37	Social Interaction
39. When she/he was 4 to 5, did she/he ever play imaginative games with another child in such a way that you could tell that each child understood what the other was pretending?		**.66**		Social Interaction
40. When she/he was 4 to 5, did she/he play cooperatively in games that required joining in with a group of other children, such as hide-and-seek or ball games?		**.82**		Social Interaction

Note: Factor loadings below .25 are suppressed

Appendix K

Factor loadings and communalities based on principal component analysis with varimax rotation for the SCQ-Lifetime for a forced three factor solution before removing all items not relating to social communication and interaction

	Factor 1	Factor 2	Factor 3	Communality
3. Has she/he ever used odd phrases or said the same thing over and over in almost exactly the same way (either phrases that she/he hears other people use or ones that she/he makes up)?		.36		.13
5. Has she/he ever got her/his pronouns mixed up (e.g. saying you or she/he for I)?	-.30	**.61**	.34	.44
7. Has she/he ever said the same thing over and over in exactly the same way or insisted that you say the same thing over and over again?		.31	.34	.20
8. Has she/he ever had things that she/he seemed to have to do in a very particular way or order, or rituals that she/he insisted that you go through?		**.68**		.51
9. Has her/his facial expression usually seemed appropriate to the particular situation, as far as you could tell?			**.51**	.33
10. Has she/he ever used your hand like a tool or as if it were a part of her/his own body (e.g. pointing with your finger or putting your hand on a doorknob to get you to open the door)?		**.61**		.36
12. Has she/he ever seemed to be more interested in parts of a toy or an object (e.g. spinning the wheels of a car), rather than in using the object as it was intended?		**.55**	.26	.47
14. Has she/he ever seemed to be unusually interested in the sight, feel, sound, taste, or smell of things or people?			**.57**	.42
16. Has she/he ever had any complicated movements of her/his whole body, such as spinning or repeatedly bouncing up and down?		.42	.37	.42
20. When she/he was 4 to 5, did she/he ever talk with you just to be friendly	**.57**		.25	.40

(rather than to get something)?				
22. When she/he was 4 to 5, did she/he ever spontaneously point at things around her/him just to show you things (not because she/he wanted them)?		.29	**.52**	.36
23. When she/he was 4 to 5, did she/he ever use gestures, other than pointing or pulling your hand, to let you know what she/he wanted?			.45	.22
24. When she/he was 4 to 5, did she/he nod her/his head to mean *yes*?			**.74**	.49
25. When she/he was 4 to 5, did she/he shake her/his head to mean *no*?			**.77**	.61
26. When she/he was 4 to 5, did she/he usually look at you directly in the face when doing things with you or talking with you?	.29	.31	.38	.30
27. When she/he was 4 to 5, did she/he smile back if someone smiled at her/him?	**.61**		.30	.37
28. When she/he was 4 to 5, did she/he ever show you things that interested him/her to engage your attention?	.31			.21
29. When she/he was 4 to 5, did she/he ever offer to share things other than food with you?	**.59**			.42
30. When she/he was 4 to 5, did she/he ever seem to want you to join in her/his enjoyment of something?	**.69**			.45
31. When she/he was 4 to 5, did she/he ever try to comfort you if you were sad or hurt?	**.72**			.48
33. When she/he was 4 to 5, did she/he show a normal range of facial expressions?	**.60**		.25	.41
36. When she/he was 4 to 5, did she/he seem interested in other children of approximately the same age whom she/he did not know?	**.64**	.25		.43
37. When she/he was 4 to 5, did she/he respond positively when another child approached her/him?	**.57**	.26		.62
38. When she/he was 4 to 5, if you came into a room and started talking to her/him without calling her/his name, did she/he usually look up and pay attention to you?	.38	**.54**		.46
39. When she/he was 4 to 5, did she/he ever play imaginative games with	.35	**.60**		.58

another child in such a way that you could tell that each child understood what the other was pretending?			
40. When she/he was 4 to 5, did she/he play cooperatively in games that required joining in with a group of other children, such as hide-and-seek or ball games?	.41	**.59**	.52

Note: Factor loadings below .25 are suppressed

Appendix L

Factor loadings and communalities based on principal component analysis with varimax rotation for the SCQ-Lifetime for a forced two factor solution before removing all items not relating to social communication and interaction

	Factor 1	Factor 2	Communality
3. Has she/he ever used odd phrases or said the same thing over and over in almost exactly the same way (either phrases that she/he hears other people use or ones that she/he makes up)?		.32	.15
5. Has she/he ever got her/his pronouns mixed up (e.g. saying you or she/he for I)?		**.67**	.46
7. Has she/he ever said the same thing over and over in exactly the same way or insisted that you say the same thing over and over again?		.45	.20
8. Has she/he ever had things that she/he seemed to have to do in a very particular way or order, or rituals that she/he insisted that you go through?		**.58**	.36
9. Has her/his facial expression usually seemed appropriate to the particular situation, as far as you could tell?		.31	.16
10. Has she/he ever used your hand like a tool or as if it were a part of her/his own body (e.g. pointing with your finger or putting your hand on a doorknob to get you to open the door)?		**.54**	.32
12. Has she/he ever seemed to be more interested in parts of a toy or an object (e.g. spinning the wheels of a car), rather than in using the object as it was intended?		**.56**	.34
14. Has she/he ever seemed to be unusually interested in the sight, feel, sound, taste, or smell of things or people?		**.54**	.33
16. Has she/he ever had any complicated movements of her/his whole body, such as spinning or repeatedly bouncing up and down?		**.61**	.40
20. When she/he was 4 to 5, did she/he ever talk with you just to be friendly (rather than to get something)?		.35	.13
22. When she/he was 4 to 5, did she/he ever spontaneously point at things around her/him just to show you things (not because she/he wanted them)?	**.64**		.43

23. When she/he was 4 to 5, did she/he ever use gestures, other than pointing or pulling your hand, to let you know what she/he wanted?	.27	**.50**	.32
24. When she/he was 4 to 5, did she/he nod her/his head to mean *yes*?		.38	.21
25. When she/he was 4 to 5, did she/he shake her/his head to mean *no*?		**.51**	.29
26. When she/he was 4 to 5, did she/he usually look at you directly in the face when doing things with you or talking with you?		**.54**	.33
27. When she/he was 4 to 5, did she/he smile back if someone smiled at her/him?	.42	.40	.34
28. When she/he was 4 to 5, did she/he ever show you things that interested him/her to engage your attention?	**.60**		.39
29. When she/he was 4 to 5, did she/he ever offer to share things other than food with you?	.38		.20
30. When she/he was 4 to 5, did she/he ever seem to want you to join in her/his enjoyment of something?	**.61**		.37
31. When she/he was 4 to 5, did she/he ever try to comfort you if you were sad or hurt?	**.64**		.41
33. When she/he was 4 to 5, did she/he show a normal range of facial expressions?	**.69**		.48
36. When she/he was 4 to 5, did she/he seem interested in other children of approximately the same age whom she/he did not know?	**.64**		.45
37. When she/he was 4 to 5, did she/he respond positively when another child approached her/him?	**.69**		.47
38. When she/he was 4 to 5, if you came into a room and started talking to her/him without calling her/his name, did she/he usually look up and pay attention to you?	**.69**		.50
39. When she/he was 4 to 5, did she/he ever play imaginative games with another child in such a way that you could tell that each child understood what the other was pretending?	**.55**		.34
40. When she/he was 4 to 5, did she/he play cooperatively in games that required joining in with a group of other children, such as hide-and-seek or ball games?	**.56**		.33

Note: Factor loadings below .25 are suppressed

Appendix M

Weak items removed from the RBS-R

3. HAND/FINGER (flaps hands, wiggles or flicks fingers, claps hands, waves or shakes hand or arm)
10. BITES SELF (bites hand, wrist, arm, lips or tongue)
11. PULLS (pulls hair or skin)
13. SKIN PICKING (picks at skin on face, hands, arms, legs or torso)